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CONTENTS: TABLE DES MATIÈRES: INHALT:

J. H. Becking, Studies on the nitrogen-fixing bacteria of the genus *Beijerinckia*. II. Mineral nutrition and resistance to high levels of certain elements in relation to soil type 297

M. I. Timonin, Effect of volatile constituents of *Scaptocoris talpa* Champ. on the growth of soil fungi 323

P. R. Hesse, Some differences between the soils of *Rhizophora* and *Avicennia* mangrove swamps in Sierra Leone 335

W. D. P. Stewart and *G. Bond*, The effect of ammonium nitrogen on fixation of elemental nitrogen in *Alnus* and *Myrica* 347

D. J. Greenwood, The effect of oxygen concentration on the decomposition of organic materials in soil 360

G. W. F. H. Borst Pauwels, Iodine as a micronutrient for plants. 377

J. W. Vail, M. S. Parry and *W. E. Calton*, Boron-deficiency dieback in pines. 393

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Corrigendum

Vol. XIV, no. 4

p. 309. Fig. 4, Abscissa. *Instead of* "PO_4^{--} concentration mg N/25 ml" *read* "PO_4^{--} concentration mg/25 ml".

p. 317. Line 18. *Instead of* "5 to 15 ppm PO_4^{--}" *read* "5 to 15 mg PO_4^{--}".

p. 382. Line 16. *Instead of* "Table 2" *read* "Table 2 (p. 379)".
Line 17. *Instead of* "(see also Figures 1 and 2)" *read* "(see also Fig. 1, p. 382, and Fig. 2, p. 380)".

p. 383. Line 4. *Instead of* "(see Table 2)" *read* "(see Table 2, p. 379)".

Line 6. *Instead of* "Figure 3" *read* "Figure 3 (p. 380)".

Plant and Soil XIV, no. 4 July 1961

STUDIES ON THE NITROGEN-FIXING BACTERIA OF THE GENUS *BEIJERINCKIA*

II. MINERAL NUTRITION AND RESISTANCE TO HIGH LEVELS OF CERTAIN ELEMENTS IN RELATION TO SOIL TYPE

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INTRODUCTION

In a previous paper [5] it was shown that nitrogen-fixing bacteria of the genus *Beijerinckia* Derx occur preferentially in tropical soils of the lateritic type.

It is well known [11 14 26 44] that laterization leads to an accumulation of iron and aluminium, and usually also of titanium [24 39] and manganese [23 24], in the upper soil layers, while there is simultaneously a leaching away of bases, particularly calcium. Their extremely high iron content gives lateritic soils a deep red colour. The iron is mostly present in the form of insoluble iron-aluminium sesquioxides or ironstone concretions. For the same reason, these soils are very low in available phosphate because it is fixed by the iron complexes. In addition to being extremely low in calcium, they are also low in other bases such as magnesium, potassium, and sodium. Therefore, lateritic soils generally have a rather low pH. Moreover, the rapid decomposition of organic matter under tropical conditions and subsequent leaching make them typically low in nitrogen. Physically, these soils are excellent: they exhibit good soil structure (ferri-crumbling) which results in nearly optimal drainage conditions and air penetration. Phosphate dressing of these soils has usually only a moderate and rather temporary effect by phosphate fixation, but nitrogen dressing produces in all cases very appreciable response in yield.

Anderson [2 3] showed that the acid, iron-rich or ironstone-con-

taining soils of South Australia and Tasmania were molybdenum-deficient, probably owing to the fixation of molybdenum. Molybdenum deficiency was also reported by Mulder [29] in the ironstone-containing, low-moor peat soils along brooklets flowing from the heath regions of Holland.

For this reason, it was considered worth-while to determine the molybdenum content of lateritic soils, especially as molybdenum is an essential trace element for biological nitrogen fixation. The molybdenum content of a senile laterite [26 44] from Bogor (Tjibinong, W. Java, Indonesia) was determined by means of the well-known Aspergillus-method [15 31 32]. By this method the soil was found to contain 0.074 ppm of available molybdenum, while an illuvial margalite soil from Ngawi (Central Java, Indonesia) derived from about the same kind of parent material contained 0.113 ppm of available molybdenum. A well-fertilized European garden soil (Wageningen, Holland) also tested, contained more than 0.4 ppm of available molybdenum. Hence, following the scale reported by Donald *et al.*[13], this lateritic soil was molybdenum poor, but not molybdenum deficient. In view of this result, it is interesting to note that Newton and Said [30] reported that a Bogor latosol of the Muara experimental field gave an improved nodulation and a considerable increase of yield in groundnuts (*Arachis hypogea*) in response to molybdenum dressing. Derx [10] showed that soil of the same Muara experimental field with a rice crop contained abundant Beijerinckia and, due to irrigation, the more alkaline soil samples contained Azotobacter in addition. The Bogor latosol (pH 4.8) from the Tjibinong Experiment Station which was tested for available molybdenum content also yielded a rich development of Beijerinckia.

It was very tempting to relate the peculiar inorganic composition of lateritic soils to the inorganic nutrition and mineral tolerance of Beijerinckia in order to provide a clue to its preferential occurrence in these soils. The present study deals with this subject. The mineral nutrition of Beijerinckia and its resistance to high levels of certain elements was tested with regard to the following elements which are of importance in lateritic soils: calcium, iron, aluminium, titanium, magnesium, manganese, and phosphate. In addition, an investigation was made of the effect of molybdenum as a trace element and of vanadium, a well-known substitute for molybdenum

in the nitrogen fixation by Azotobacter [7 17]. Finally, the effect of copper and zinc as trace elements on the nitrogen fixation of Beijerinckia was studied.

EXPERIMENTAL

Several Beijerinckia strains were used in these experiments, but in most use was made only of one, *Beijerinckia indica* str. Hawaii 3/1, freshly isolated from a "Humic Latosol" [24 38] of pH 5.1 from Kaneohe (Oahu, Hawaii Islands) * which showed all the features characteristic of the genus Beijerinckia. This is a rather fast-growing strain: in stationary culture the maximum yield by nitrogen fixation was attained and most of the glucose (2% w/v) was consumed after 35 days incubation at 30°C. Some of the distinguishing features of slow- and fast-growing strains will be reported in a subsequent paper.

For comparison's sake, certain Azotobacter strains were included in some of the present experiments. Details about these will be given in the appropriate sections.

Growth of Beijerinckia was determined as nitrogen fixation in a nitrogen-free basic medium of the following composition: distilled water (from an all-glass pyrex apparatus) 1000 ml, glucose 20.0 g, K_2HPO_4 0.8 g, KH_2PO_4 0.2 g, $MgSO_4.7H_2O$ 0.5 g, $FeCl_3.6H_2O$ 0.05 g, $MnSO_4.4H_2O$ 0.005 g, $ZnSO_4.6H_2O$ 0.005 g, $CuSO_4.5H_2O$ 0.004 g, and $Na_2MoO_4.2H_2O$ 0.005 g; pH 6.9. The Azotobacter strains were grown in a similar medium, but the phosphate was replaced by K_2HPO_4 1.0 g, and calcium carbonate 4.0 g was added since most Azotobacter strains require calcium for growth. In the experiments concerning the influence of the various elements, the element under investigation was omitted from the basic medium.

In the trace-element experiments all glassware was thoroughly cleaned by washing several times with hot water and organic detergent (Teepol), followed by treatment with sulphuric acid (30%) and strong alkali (40%) or, alternatively, with a solution (2%) of di-sodium-ethylenediamine-tetra-acetic acid (EDTA) in distilled water, and finally rinsed with pyrex-distilled water.

The medium was pipetted in 25-ml aliquots into 100-ml erlenmeyer flasks and sterilized for 20 minutes at 110°C. To prevent precipitation of the element to be tested during the sterilization process, it was added from sterile stock solutions to the erlenmeyer flasks containing the sterile basic medium. Long-necked erlenmeyer flasks were used which were covered with small glass beakers instead of cotton plugs.

The flasks were inoculated with cells from a 6- to 7-day old Beijerinckia or a 3-day old Azotobacter agar slant culture grown on nitrogen-free mineral glucose agar. In the experiments with trace elements, the element to be tested was omitted from the agar slant medium in order to produce cells sufficiently

* For this soil the author is indebted to Dr G. Donald Sherman, Department of Soils and Agricultural Chemistry, University of Hawaii.

low in this element. The cultures were harvested after an incubation period of 35 days for Beijerinckia and of 14 days for Azotobacter at 30°C. Nitrogen fixation was determined by nitrogen increase (*i.e.* the initial amount of nitrogen in the medium was substracted) according to the convential micro-Kjeldahl procedure following the modification of Yuen and Pollard [46].

All figures presented are the average values from duplicate experiments, except those of the phosphate and trace elements in which four replicates were used.

In all the work reported below, the organisms were grown on nitrogen-free media, where growth is dependent upon nitrogen fixation; hence the two terms "growth" and "nitrogen fixation" are used interchangeably.

RESULTS

The results of experiments on the following elements are presented below.

(a) *Calcium*

Ten different *Beijerinckia indica* strains were tested, including the *Beijerinckia mobile* type strain of Derx [10]. In addition, ten different Azotobacter strains were tested for sake of comparison. The latter strains belonged to the species: *A. chroococcum*, *A. vinelandii*, and *A. agile*. All the strains used were isolated from soil except the *Azotobacter agile* strains which were isolated from water.

Since all experiments with the Beijerinckia and Azotobacter strains gave results showing similar differences between the species, only a few typical experiments with each species will be reported.

TABLE 1

Nitrogen fixation (mg N/25 ml medium) in relation to calcium content of the medium (Initial pH = 6.9)

Species	No Ca added (control)	500 µg Ca * per 25 ml medium
Beijerinckia indica str. Hawaii 3/1	6.2	5.9
,, ,, str. Yugoslavia 3b/1a	6.4	6.4
,, ,, str. Trinidad 17/1	6.0	6.0
Beijerinckia mobile str. 1, Java	5.0	5.4
Azotobacter chroococcum str. 1, Delft, Holland . . .	0.1	4.3
Azobacter agile str. 19, Rhine water, Wageningen, Holland	4.6	6.0
Azotobacter vinelandii str. A 18/1, Bolivia	2.0	5.7

* Applied as $CaCl_2.6H_2O$ (Merck, A.R.).

As is evident from Table 1, the nitrogen fixation in the *Beijerinckia indica* and *mobile* strains was essentially the same in the presence or absence of calcium. In contrast to this, the Azotobacter strains showed a considerable response to calcium which varied from one species to another. The most sensitive to calcium deficiency were the *Azotobacter chroococcum* strains in which practically no nitrogen fixation occurred in the absence of this element. However, the *Azotobacter vinelandii* strains used produced moderate nitrogen fixation in media without added calcium, and in particular the *Azotobacter agile* strains produced considerable nitrogen fixation without calcium addition. Apparently, for the latter species, calcium is either not essential but merely stimulatory for growth, or these strains have far lower calcium requirements and the ability to utilize the extremely low levels of it which are present as a contamination in the basic nutrient solution.

In a previous study by the present author [22], attention was drawn to the fact that calcium carbonate seemed to inhibit nitrogen fixation in Beijerinckia. The "calciphobe" behaviour of this species was already noted in 1936 by Altson [1], who isolated this organism from Malayan soils. Our experiments showed that Beijerinckia cultures, streaked out directly from the enrichment medium on a nitrogen-free glucose agar medium containing 2% calcium carbonate, produced good growth. Subsequent transfers onto similar plates, however, grew slowly and poorly or not at all, but others transferred onto the same medium in which the calcium carbonate was either omitted or replaced by 0.005% $CaCl_2.2H_2O$ showed excellent development. At that time no explanation could be given for this phenomenon, especially as calcium carbonate is a rather inert, insoluble substance, which is generally only used for the detection or neutralization of acid produced by micro-organisms. Experiments which will be reported later in this paper gave evidence, however, that Beijerinckia had a larger trace-element requirement than Azotobacter. For this reason, a possible explanation for the observed phenomenon may be that the sterilization of the medium in the presence of calcium carbonate leads to a lowering of the available trace-element content of the medium because the latter is a powerful agent for removing trace elements from nutrient solutions (Steinberg [42 43]). Although not definitely proved, this opinion is strengthened by the observation that an

addition of sterile calcium carbonate powder to previously sterilized media yielded no growth inhibition.

(b) *Iron*

The effect of iron at varying concentrations on the nitrogen fixation of the *Beijerinckia indica* str. Hawaii 3/1 is presented in Figure 1. The Figure shows that there was very little nitrogen

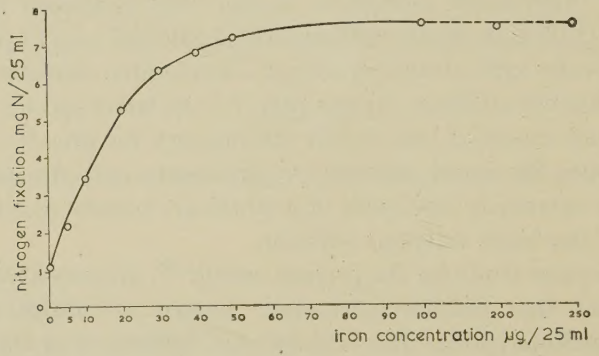

Fig. 1. Nitrogen fixation of *Beijerinckia indica* strain Hawaii 3/1 in relation to the iron content of the medium. (Initial pH = 6.9).

fixation in the absence of added iron. The small amount of growth obtained in the latter case was probably due to the trace amounts of iron present as contamination in the basic medium or to the introduction of iron with the inoculum. Maximum nitrogen fixation was reached at the relatively high iron level of 50 to 100 µg Fe per 25 ml, *i.e.* 2 to 4 ppm. A further increase in iron concentration did not increase nitrogen fixation. Half-maximal nitrogen fixation – a reliable method for comparing quantitative requirements in different strains – was obtained at an iron concentration of 12 to 15 µg Fe per 25 ml, *i.e.* 0.5 to 0.6 ppm.

In addition, the effect of high iron levels on nitrogen fixation of Beijerinckia was tested at three pH levels and compared with Azotobacter under similar conditions.

In these experiments use was made of stock solutions of $FeSO_4.7H_2O$ (Merck, A. R.) containing 500 µg and 2000 µg Fe per ml. When prepared in distilled water these solutions were strongly acid (pH 3.5 to 4.0) and they therefore had to be neutralized before use. This caused much precipitation, so that the concentrations given are only approximate. The pH values given

are the initial values only. During growth of Beijerinckia acid was produced and the pH value of media at initial pH 7.5 or 7.1 was lowered to pH 5.0, and that of pH 5.0 to pH 3.0. With Azotobacter the medium stayed at about the same pH value during growth. Buffering the media by increasing the phosphate concentration was inadvisable because higher phosphate concentrations were found to limit growth of Beijerinckia (see Table 11) and organic buffers could not be used as they influence nitrogen fixation owing to their nitrogen content.

The results presented in Table 2 are somewhat variable owing to pH changes in the medium caused by the addition of iron and, in the case of Beijerinckia, also by acid production. In spite of this, some general conclusions can be drawn.

TABLE 2

Nitrogen fixation (mg N 25 ml medium) in *Beijerinckia indica* str. Hawaii 3/1 and *Azotobacter chroococcum* str. 1 Delft at high iron levels and at different pH values

Fe-concentration * μg/25 ml medium	*Beijerinckia indica* str. Hawaii 3/1			*Azotobacter chroococcum str.* 1 Delft	
	Initial pH			Initial pH	
	7.5	7.1	5.0	7.5	7.1
250	8.6	8.9	6.1	5.4	5.1
500	8.7	9.1	6.2	3.5	6.2
1000	8.8	8.8	6.8	3.6	3.8
2000	8.9	9.0	7.3	5.3	4.8
5000	9.1	7.9	7.8	1.6	1.3

* Applied as $FeSO_4.7H_2O$ (Merck, A.R.).

In Beijerinckia nitrogen fixation at pH 7.5 and 7.1 (final pH 5.0) was not influenced by high iron concentrations. In the pH series pH 5.0 (final pH 3.0 to 3.2) there was some reduction in nitrogen fixation. However, this was primarily due not to iron inhibition but to a pH effect, for in this series the final pH limited growth (Becking [5]).

In contrast to this in Azotobacter at a pH of 7.5 or 7.1 and at an iron concentration of 5000 μg per 25 ml (200 ppm) nitrogen fixation was considerably reduced. The pH of 5.0 was not tested because at this pH Azotobacter produces no growth irrespective of the iron concentration. These experiments show that Azotobacter is far more susceptible to high iron levels than Beijerinckia.

(c) *Aluminium*

The effect of trace amounts of aluminium on the nitrogen fixation of *Beijerinckia indica* str. Hawaii 3/1 is presented in Table 3.

TABLE 3

Nitrogen fixation in *Beijerinckia indica* str. Hawaii 3/1 in relation to the aluminium content of the medium (Initial pH = 6.9).

Aluminium * concentration in μg/25 ml medium	Nitrogen fixation mg N/25 ml medium
0	7.4
5	7.4
20	7.5
40	7.2
50	7.5
100	7.2
200	6.8

* Applied as $AlCl_3$ anhydr. (Merck, A.R.).

As shown, Al concentrations up to 100 μg per 25 ml (4 ppm) did not affect nitrogen fixation. Only at 200 μg Al per 25 ml (8 ppm) was there a very small reduction in nitrogen fixation.

In experiments where iron was omitted from the basic medium and replaced by an equivalent amount of Al, the yields were equal to those obtained without added iron and aluminium. Hence, aluminium cannot replace iron in nitrogen fixation.

The effect of large concentrations of aluminium on the nitrogen fixation of two Beijerinckia strains and one Azotobacter strain is presented in Table 4.

As in the iron experiments, the Al stock solutions containing 100 and 500 μg Al per ml were very acid (pH 3.0 to 3.5) and therefore were neutralized before use. This caused a large precipitation in consequence of which the Al concentrations given are only approximate. For this reason the results are somewhat variable and reflect pH differences in the media.

The figures clearly show that nitrogen fixation in the *Beijerinckia indica* str. Hawaii 3/1 was maximal even at very high aluminium levels (2000 μg Al per 25 ml = 80 ppm) at pH 7.5 or 7.1 (final pH 5.0). But at pH 5.0 (final pH 3.0 to 3.2) there was a considerable

TABLE 4

Nitrogen fixation (mg N/25 ml medium) by *Beijerinckia indica* str. Hawaii 3/1, *Beijerinckia mobile* str. 1 Java, and *Azotobacter chroococcum* str. 1 Delft at high aluminium levels and various pH values

Al-concentration * μg/25 ml medium	*Beijerinckia indica* str. Hawaii 3/1			*Beijerinckia mobile* str. 1 Java			*Azotobacter chroococcum* str. 1 Delft	
	Inital pH			Initial pH			Initial pH	
	7.5	7.1	5.0	7.5	7.1	5.0	7.5	7.1
0	6.3	6.0	5.8	3.7	4.2	5.8	5.1	5.1
100	6.5	6.2	3.1	–	–	–	5.2	5.2
200	6.9	5.8	2.5	–	–	–	4.7	4.9
250	6.5	6.9	2.1	3.8	3.5	5.4	4.4	3.3
500	7.2	6.3	2.2	4.1	3.9	5.4	4.6	3.1
1000	8.0	7.2	1.5	5.8	5.5	5.9	3.8	1.9
2000	7.6	7.3	2.6	5.6	7.0	6.7	1.2	0.4

* Applied as $Al_2(SO_4)_3 \cdot 18\ H_2O$ (Union Chimique Belge, A.R.).

reduction at nitrogen fixation, especially at the higher aluminium concentrations. This effect was primarily due to the presence of aluminium, since in the absence of aluminium nitrogen fixation was only slightly reduced at acid reaction.

In the *Beijerinckia mobile* str. 1 Java, at all aluminium concentrations more nitrogen was fixed at an acid reaction (initial pH 5.0, final pH 3.0 – 3.2) than at an alkaline reaction. This was, however, due to the fact that this strain is extremely acid-tolerant, showing optimal growth at pH 3.5 (Becking [5]). Apparently with this strain the combined action of high aluminium concentration and low pH did not inhibit nitrogen fixation.

In the *Azotobacter chroococcum* strain tested at both pH 7.5 and 7.1, nitrogen fixation was strongly reduced at the highest aluminium levels tested, *i.e.* at 2000 μg Al per 25 ml = 80 ppm. At pH 7.1 this effect was somewhat more pronounced than at pH 7.5. Hence, Beijerinckia seems to be far more tolerant than Azotobacter of high aluminium concentrations even at low pH.

(d) *Titanium*

The effects of traces and of high concentrations of this element on nitrogen fixation were tested. As evident from Table 5, titanium as trace element, *i.e.* at a concentration of 30 μg per 25 ml = 1.2 ppm, did not influence nitrogen fixation in three *Beijerinckia indica*

TABLE 5

Nitrogen fixation (mg N/25 ml medium) in relation to the titanium content of the medium
(Initial pH = 6.9)

Species	0 Ti (control)	30 μg Ti * per 25 ml medium
Beijerinckia indica str. Hawaii 3/1	6.2	6.3
,, ,, str. Yugoslavia 3b/1a	6.4	6.5
,, ,, str. Trinidad 17/1	6.0	5.5
Beijerinckia mobile str. 1 Java	5.0	5.6

* Applied as $Ti_2(SO_4)_3$ (British Drug Houses, Lab. Reagent).

TABLE 6

Nitrogen fixation (mg N/25 ml medium) in *Beijerinckia indica* str. Hawaii 3/1 at high titanium concentrations at various pH values

Ti-concentrations * μg/25 ml medium	Initial pH		
	7.5	7.1	5.0
250	8.7	8.3	7.1
500	8.3	8.1	7.5
1000	8.4	8.4	7.8
2000	8.1	8.4	8.4
4000	–	–	8.5

* Applied as $Ti_2(SO_4)_3$ (British Drug Houses, Lab. Reagent).

strains and one *Beijerinckia mobile* strain. In Table 6 the effect of very high levels of titanium on nitrogen fixation of the *Beijerinckia indica* str. Hawaii 3/1 at various pH values is presented. It is clear that there was no reduction in nitrogen fixation whatever the titanium concentration or pH value. Hence, Beijerinckia is also very tolerant of high titanium concentrations at low pH.

(e) *Magnesium*

In Figure 2 the magnesium requirement of the *Beijerinckia indica* str. Hawaii 3/1 is presented. The Figure shows that this strain growing in a basic medium with inorganic phosphate reached maximal nitrogen fixation at a magnesium level of 400 to 500 μg Mg per 25 ml (16 to 20 ppm) and half-maximal nitrogen fixation at 100 μg Mg per 25 ml (4 ppm). This is a rather considerable magnesium requirement and is quite comparable to the figures given in

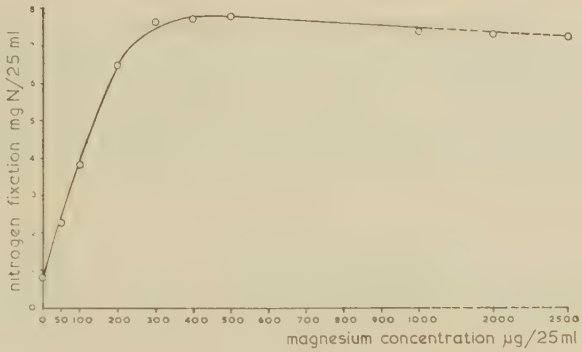

Fig. 2. Nitrogen fixation of *Beijerinckia indica* strain Hawaii 3/1 in relation to the magnesium content of the culture solution. (Initial pH = 6.9).

the literature for *Azotobacter* under identical conditions [19 45]. It must be emphasized that by using organic phosphate compounds other values for magnesium requirement may be obtained as shown by Jensen [19].

(f) *Manganese*

The effect of manganese both in traces and at very high concentrations was tested at various pH values. Figure 3 gives the effect of a range of low levels of manganese on the nitrogen fixation of the *Beijerinckia indica* str. Hawaii 3/1. There was no apparent manganese requirement and increasing amounts of manganese tended

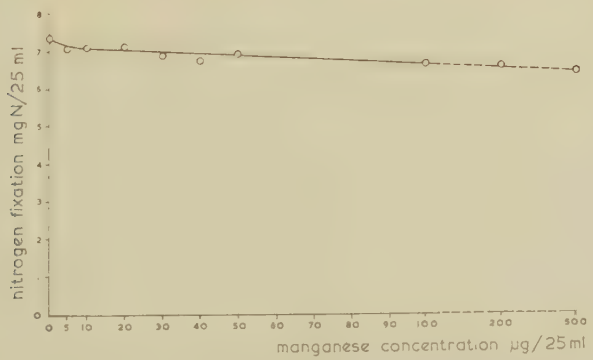

Fig. 3. Nitrogen fixation of *Beijerinckia indica* str. Hawaii 3/1 in relation to the manganese content of the culture solution. (Initial pH = 6.9).

to decrease nitrogen fixation very slightly. At very high concentrations manganese had no effect on nitrogen fixation at pH 7.5 or 7.1 (final pH 5.0), but there was a reduction of about 50 per cent at pH 5.0 (final pH 3.0 to 3.2) at the highest Mn levels tested, *i.e.* 10,000 μg Mn per 25 ml (400 ppm); see Table 7.

TABLE 7

Nitrogen fixation (mg N/25 ml medium) by *Beijerinckia indica* str. Hawaii 3/1 at high manganese concentrations at various pH values

Mn-concentrations * μg/25 ml medium	Initial pH		
	7.5	7.1	5.0
500	8.3	7.8	6.5
1000	8.3	7.9	6.3
2500	8.5	8.0	6.4
5000	7.7	7.9	5.5
10,000	–	–	3.7

* Applied as $MnSO_4.4H_2O$ (Merck, A.R.).

TABLE 8

Nitrogen fixation (mg N/25 ml medium) in two *Azotobacter chroococcum* strains in relation to the manganese content of the medium. (Initial pH = 6.9)

Mn-concentration * μg/25 ml medium	*Azotobacter chroococcum* str. 1, Delft, Holland	*Azotobacter chroococcum* str. 5, Ngawi, Java
0	5.9	0.9
10	5.4	2.1
500	0.2	5.5

* Applied as $MnSO_4.4H_2O$ (Merck, A.R.).

The influence of manganese on the nitrogen fixation of two *Azotobacter chroococcum* strains was investigated and the results are presented in Table 8. The *Azotobacter chroococcum* strain isolated from Dutch soil showed no apparent manganese requirement, while the *Azotobacter chroococcum* strain from the tropical soil rich in manganese showed very poor growth with no manganese addition and maximal nitrogen fixation with 500 μg Mn per 25 ml (20 ppm).

(g) *Phosphate*

The effect of low and high concentrations of phosphate on growth and nitrogen fixation was investigated with three Beijerinckia

strains (two *B. indica* strains and one *B. mobile* strain) and with two *Azotobacter chroococcum* strains. All strains were grown in the same medium and incubated together. For this reason the basic medium used for Beijerinckia was supplied with calcium ($CaCl_2 . 6H_2O$, 0.05 g per litre) since *Azotobacter chroococcum* needs calcium for growth.

The phosphate was supplied as K_2HPO_4 (Merck, A. R.). At the higher levels, a phosphate buffer containing K_2HPO_4 and KH_2PO_4 in the ratio of 4 : 1 was used to avoid excessive alkaline reaction of the medium .The KH_2PO_4 was also Merck, A. R. quality.

The effect of increasing amounts of phosphate on the nitrogen fixation of *Beijerinckia indica* str. Hawaii 3/1 is shown in Figure 4. Maximal nitrogen fixation in this strain was already obtained at the phosphate level of 2 mg PO_4^{--} per 25 ml ,*i.e.* 80 ppm.

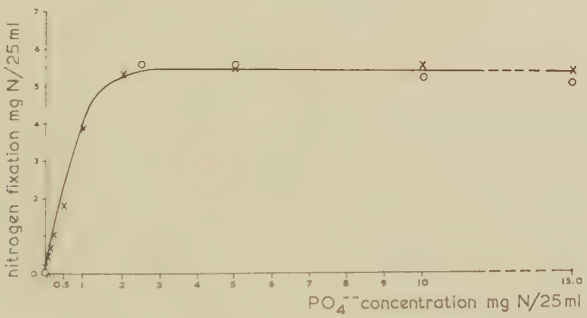

Fig. 4. Nitrogen fixation of *Beijerinckia indica* str. Hawaii 3/1 in relation to the phosphate content of the culture solution. × and ○ denote two separate experimental series. (Initial pH = 6.9)

In Table 9 a comparison was made of the nitrogen fixation in relation to the phosphate concentration of the medium between some Beijerinckia and Azotobacter strains. The results show that Beijerinckia started to fix nitrogen at much lower phosphate levels than Azotobacter. In the latter species nitrogen fixation was completely checked below a phosphate level of 0.5 mg PO_4^{--} per 25 ml (20 ppm).

In a second series the effect of high phosphate concentrations on the nitrogen fixation of Beijerinckia and Azotobacter was tested. As Table 10 shows, there was a considerable reduction in nitrogen

TABLE 9

Nitrogen fixation (mg N 25 ml medium) in relation to the phosphate * content of the medium (Initial pH = 6.9)

Species	mg PO_4^{--}/25 ml medium									
	0	0.05	0.1	0.25	0.5	1.0	2.0	2.5	5.0	10.0
Beijerinckia indica str. Hawaii 3/1	0.1	0.4	0.6	1.2	2.1	3.2	5.3	6.6	7.3	7.1
,, ,, str. 8, Bogor, Java	0.1	0.5	0.7	1.0	1.8	3.9	5.3	–	5.6	5.7
Beijerinckia mobile str. 1, Bogor, Java	0.2	0.5	0.6	0.9	1.4	2.2	5.4	5.5	6.0	5.6
Azotobacter chroococcum str. 1, Delft, Holland	0	0	0	0	0.1	1.4	2.9	3.6	7.1	7.2
Azotobacter chroococcum str. 5, Ngawi, C. Java	0	0	0	0	0	0.6	0.9	1.3	1.9	3.0

* Applied as K_2HPO_4 (Merck, A.R.).

TABLE 10

Nitrogen fixation (mg N/25 ml medium) in relation to the phosphate * content of the medium (Initial pH = 6.9)

Species	mg PO_4^{--}/25 ml medium							
	5	10	20	40	60	100	150	200
Beijerinckia indica str. Hawai 3/1	7.2	6.6	5.2	6.4	5.7	5.0	5.1	4.6
,, ,, str. 8, Bogor, Java	5.6	5.3	5.2	5.2	5.3	5.5	5.4	5.7
Beijerinckia mobile str. 1, Bogor, Java	6.2	5.8	5.0	4.7	4.2	4.1	4.3	4.0
Azotobacter chroococcum str. 1, Delft, Holland	7.1	7.5	7.3	5.8	1.1	0.3	0.4	0.5
Azotobacter chroococcum str. 5, Ngawi, C. Java	6.7	6.1	5.2	4.3	1.5	0.1	0.6	0.1

* Applied as K_2HPO_4 (Merck, A.R.).

fixation in Azotobacter at phosphate levels higher than 40 mg PO_4^{--} per 25 ml (1,600 ppm). Beijerinckia, however, was far less susceptible to high phosphate concentrations, for at a concentration ten times higher, *i.e.* 400 mg PO_4^{--} per 25 ml = 16,000 ppm, nitrogen fixation was only reduced 35 to 39 per cent in the *Beijerinckia indica* str. Hawaii 3/1 and in the *Beijerinckia mobile* str. 1 Java, and not at all in the *Beijerinckia indica* str. 8, Bogor, Java.

The upper limit of phosphate tolerance in Beijerinckia was determined in a third series summarized in Table 11. This Table shows that only a concentration of 4.0% potassium phosphate (which is equivalent to *ca* 580 mg PO_4^{--} per 25 ml = 23,200 ppm) entirely

TABLE 11

Nitrogen fixation at different phosphate concentrations of the medium by *Beijerinckia indica* str. Hawaii 3/1						
Potassium phosphate *	0.1%	0.5%	1.0%	2.0%	4.0%	5.0%
PO_4^{--}, mg/25 ml medium	14.4	72	144	288	576	720
Nitrogen fixation, mg N/25 ml medium	6.8	6.1	5.1	5.1	0	0

* Phosphate buffer, K_2HPO_4 : KH_2PO_4 = 4 : 1 (w/w), of pH 7.1; percentages w/v; both chemicals (Merck, A.R.).

inhibited nitrogen fixation in the *Beijerinckia indica* str. Hawaii 3/1.

(h) *Molybdenum* and *vanadium*

The effect of trace amounts molybdenum and vanadium on nitrogen fixation was tested in two *Beijerinckia indica* strains and in a typical *Azotobacter vinelandii* strain.

The Beijerinckia strains were grown in a medium with glucose and the Azotobacter strain with ethanol as sole carbon sources. Ethanol, which was easily freed from molybdenum impurities by redistillation in a pyrex apparatus, could not be used as a carbon source for Beijerinckia because the latter grew poorly on it. Since glucose proved to be rather difficult to purify, several commercial batches of glucose were tested with the Aspergillus technique [15 31 32] for their molybdenum content. A product obtained from the Chinine Company, Amsterdam, was the only one sufficiently low in molybdenum (it contained 0.0012 ppm Mo) to be used in these experiments without further purification.

In the experiments with Azotobacter the basic medium used was supplied with calcium in the form of calcium carbonate (0.4%) since *Azotobacter vinelandii* requires calcium for maximal growth. The carbonate was precipitated from $CaCl_2$ with thrice recrystallized Na_2CO_3.

Beijerinckia indica str. Hawaii 3/1 was isolated from a "Humic Latosol" [24 38] (pH 5.1) from Kaneohe, Oahu, Hawaii Islands; *Beijerinckia indica* str. Tanganyika 4/1 from an illuvial ("Mbuga") soil [27] (pH 7.1) of Ukiriguru Research Station, Lake Province, Tanganyika and the *Azotobacter vinelandii* strain from clay soil (pH 7.6) of Trinidad-Beni, Bolivia.

The results obtained with the Beijerinckia strains and the Azotobacter strain are presented in Figures 5 and 6 respectively.

As evident from Figure 5, nitrogen fixation in the *Beijerinckia indica* str. Hawaii 3/1 increased up to a molybdenum concentration

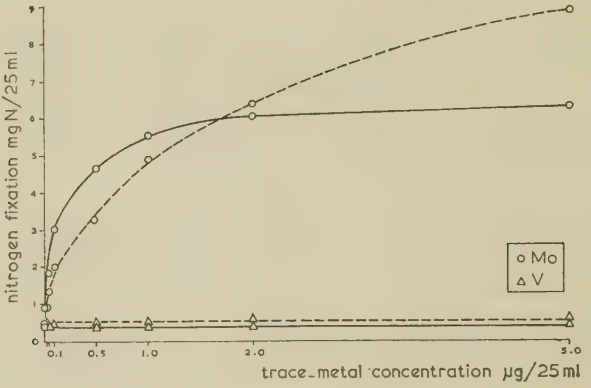

Fig. 5. Nitrogen fixation of *Beijerinckia indica* str. Hawaii 3/1 and *Beijerinckia indica* str. Tanganyika 4/1 in relation to molybdenum and vanadium content of the culture solution. Interrupted line: *B. indica* str. Hawaii 3/1; continuous line: *B. indica* str. Tanganyika 4/1. (Initial pH = 6.9).

of 5.0 μg per 25 ml (0.2 ppm), while half-maximal nitrogen fixation was reached at approximately 0.85 μg Mo per 25 ml (0.034 ppm). In the *Beijerinckia indica* str. Tanganyika 4/1, however, maximal and half-maximal nitrogen fixation were reached at 2.0 μg Mo per 25 ml (0.08 ppm) and 0.1 μg Mo per 25 ml (0.004 ppm) respectively. Vanadium could not replace molybdenum in the nitrogen fixation of these two strains. To confirm the more general validity of this

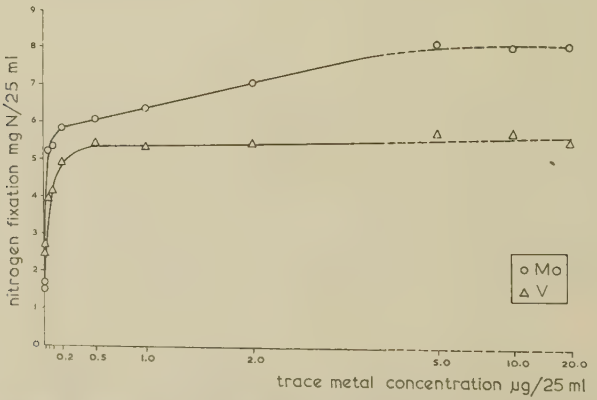

Fig. 6. Nitrogen fixation of *Azotobacter vinelandii* str. Bolivia A 18/1 in relation to molybdenum and vanadium content of the culture solution. (pH = 7.3).

observation for the genus Beijerinckia, twenty-five other Beijerinckia strains, assigned to different species (*Beijerinckia indica* [9 41], *B. mobile* [10], *B. lacticogenes* [21], *B. fluminensis* [12]) were tested in this respect. In none of these strains could vanadium replace molybdenum in nitrogen fixation.

The results obtained with the *Azotobacter vinelandii* str. 8 Bolivia are presented in Figure 6. From this Figure it is clear that even extremely low molybdenum concentrations beneficially affect nitrogen fixation in this strain. Although the molybdenum requirement for maximal nitrogen fixation was about the same as in the Beijerinckia strains, half-maximal nitrogen fixation was obtained at a much lower concentration, *viz* at 0.01 μg per 25 ml (0.0004 ppm). In addition, vanadium was able to replace molybdenum in nitrogen fixation, but its maximum effect was only about two-thirds of that produced by molybdenum. However, the vanadium concentration producing maximal nitrogen fixation was ten times lower than that of molybdenum, *viz* 0.5 μg V per 25 ml (0.02 ppm) against 5.0 μg Mo per 25 ml (0.2 ppm).

Many other Azotobacter strains belonging to different species were tested in respect of the replaceability of molybdenum by vanadium. In contrast to the general opinion that vanadium can act as a substitute for molybdenum in the nitrogen fixation of Azotobacter, it was observed that vanadium did not have this action in all the *Azotobacter agile* strains tested (in sharp contrast to *A. vinelandii*) nor indeed in some typical *Azotobacter chroococcum* strains. The results of these experiments will be reported in a separate paper.

(i) *Copper and zinc*

The influence of copper and zinc as trace elements (1 ppm) on nitrogen fixation of the *Beijerinckia indica* str. Hawaii 3/1 was tested. The results (Table 12) show that the copper-free and the zinc-free cultures yielded amounts of fixed nitrogen 24 and 12 per cent lower, respectively, than the controls. Although these differences may not be considered very great, they are highly significant, due to the small deviations in the four replicates.

TABLE 12

Nitrogen fixation in *Beijerinckia indica* strain Hawaii 3/1 in relation to the copper and zinc contents of the medium. (Initial pH = 6.9)

Trace-element * concentration μg/25 ml medium		mg N fixed per 25 ml medium	Increase
Cu	0	5.2	–
	25	6.9	24%
Zn	0	6.2	–
	25	7.1	12%

* Applied as $CuSO_4.5\,H_2O$ and $ZnSO_4.7\,H_2O$. (Merck, A.R.).

DISCUSSION

Lateritic soils, the preferential habitat of representatives of the genus *Beijerinckia*, are extremely poor in calcium, but very rich in iron and aluminium, and usually also in titanium and manganese. In addition, they are very low in available phosphate due to the phosphate fixation by the abundant iron complexes present. Moreover, by virtue again of their high iron content, lateritic soils are low in available molybdenum, but are not molybdenum deficient. Owing to their eluvial character, these soils are not only low in calcium but also in other bases such as sodium, potassium, and magnesium, and they have in consequence a rather low pH.

A correlation was found between the mineral composition of lateritic soils and the mineral requirements of Beijerinckia.

First, the extremely low calcium level in lateritic soils is of interest in view of the fact that Beijerinckia, in contrast to Azotobacter, requires no calcium for growth and nitrogen fixation. However, the calcium requirement generally ascribed to Azotobacter was not shown by all the strains of this genus tested. Indeed, all the *Azotobacter chroococcum* strains tested showed practically no nitrogen fixation without calcium addition, but the *Azotobacter vinelandii* strains and the *Azotobacter agile* strains fixed significant amounts of nitrogen in the absence of added calcium. In particular in *Azotobacter agile*, calcium seems not to be essential but only stimulatory for nitrogen fixation, confirming an earlier observation of Norris and Jensen [36 37].

In previous experiments (Altson [1], Kluyver and Becking [22])

it had been observed that Beijerinckia was "*calciphobe*" with respect to calcium carbonate but not to calcium chloride. This behaviour could be partially explained by the observation reported above that Beijerinckia needs far more trace elements than Azotobacter and the fact that sterilization of media in the presence of calcium carbonate removes trace elements from the solution as shown by Steinberg [42 43]. This conclusion is strengthened by the observation that the addition of sterile calcium carbonate to a medium previously sterilized had no inhibitory effect on nitrogen fixation.

The rather high iron requirement of Beijerinckia compared to Azotobacter can be correlated with the fact that its preferential habitat is in iron-rich latosols. In the Beijerinckia strain tested, maximal nitrogen fixation was attained at 2 to 4 ppm Fe, whereas half-maximal nitrogen fixation was at 0.5 to 0.6 ppm Fe. Burk, Lineweaver, and Horner [8] and Horner and Burk [16] extensively studied the iron requirements of Azotobacter and observed maximal nitrogen fixation at 0.02 to 0.5 ppm Fe (0.0004 to 0.01 millimole) and half-maximal nitrogen fixation at 0.006 to 0.008 ppm Fe (1.1 to 1.4×10^{-4} millimole). The latter authors concluded that the demonstration of a definite iron requirement in Azotobacter necessitated an absolute purification of the medium with respect to iron rather than an addition of iron.

Also the observation that Beijerinckia is extremely resistant to high iron levels, even at low pH, is consistent with the high iron levels of lateritic soils.

Aluminium, also present in large quantities in lateritic soils, was found to be neither essential nor stimulatory in nitrogen fixation of Beijerinckia. At the very high concentration of 80 ppm, aluminium did not influence the nitrogen fixation in Beijerinckia at pH 7.5 or 7.1 (final pH 5.0), but it had some inhibitory effect when low pH (initial pH 5.0, final pH 3.0 to 3.2) was combined with high aluminium concentrations. This effect was, however, absent in the very acid-tolerant *Beijerinckia mobile* str. 1 which irrespective of pH and aluminium concentration fixed more nitrogen at low than at high pH. This was primarily due to the fact that this strain showed optimal nitrogen fixation at pH 3.5 and a sharp decline of nitrogen fixation towards the alkaline side[5]. In relation to the above-mentioned results on the aluminium tolerance of Beijerinckia, it is worth while mentioning that Katznelson [20]

has already shown that the survival of Beijerinckia (*Azotobacter indicum*) in acid soil filtrates containing large amounts of soluble aluminium and iron is much greater than that of Azotobacter under similar conditions. In addition, he showed that Beijerinckia could produce growth in a medium containing 75 to 100 ppm $Al_2(SO_4)_3$ even at a pH 4.0.

Titanium, a very common element in most lateritic soils, was shown to be neither essential for nitrogen fixation as trace element nor inhibitory at high levels. This great tolerance is certainly an advantage for Beijerinckia when growing in titaniferous lateritic soils [24 39].

Magnesium was tested since it is an essential element for growth in bacteria. Jensen [19] has already studied the magnesium requirements both of Azotobacter and of Beijerinckia. He observed that in a medium containing inorganic phosphate the two Beijerinckia strains tested had a far lower magnesium requirement than Azotobacter, *i.e.* for optimal nitrogen fixation Beijerinckia required 0.4 to 1.0 ppm Mg and *Azotobacter chroococcum* 20 to 40 ppm Mg. We tested the magnesium requirement of the *Beijerinckia indica* str. Hawaii 3/1 and found that for maximal and half-maximal nitrogen fixation magnesium levels of 20 ppm and 4 ppm, were required. This is indeed a very high magnesium requirement for a Gram-negative bacterium and is comparable to that observed in Azotobacter. Hence, a lower magnesium requirement in Beijerinckia is not an invariable character of all strains. The high magnesium requirement of our strain, however, might be related to its origin since it was isolated from a relatively magnesium-rich latosol in the Hawaii Islands which was derived from a very magnesium-rich olivine basalt [40] parent material. The lower magnesium requirement of Beijerinckia as compared with Azotobacter which was observed by Jensen [19], is undoubtedly an adaptation to lateritic soils, because these soils are generally nearly as low in magnesium as in calcium.

Manganese, also commonly found in lateritic soils, was tested. Beijerinckia did not apparently need manganese nor did an *Azotobacter chroococcum* strain isolated from a manganese-poor soil of the temperate region. However, an *Azotobacter chroococcum* strain isolated from a manganese-rich tropical soil showed a definite manganese requirement and maximal nitrogen fixation at a manganese level of 20

ppm. Manganese at very high levels had no effect on nitrogen fixation of Beijerinckia at pH 7.5 or 7.1 (final pH 5.0), but there was about 50 per cent reduction in nitrogen fixation at a manganese level of 400 ppm at pH 5.0 (final pH 3.0 to 3.2). Since the latter conditions are rather extreme for lateritic soils, it may be concluded that *Beijerinckia* is rather tolerant of the nigh manganese levels of its natural environment.

Phosphate is generally available in very low concentrations in lateritic soils because of phosphate fixation by iron complexes which are abundantly present in them. This fact agrees with the observation that Beijerinckia was able to utilize extremely low levels of phosphate – even the minute amounts present as contamination in the basic medium – while Azotobacter produced no growth below a phosphate concentration of 20 ppm. Maximal nitrogen fixation, however, was not found to occur at widely differing phosphate concentrations in Beijerinckia and in Azotobacter. In the former a concentration of 2 to 5 mg PO_4^{--} per 25 ml (80 to 200 ppm) gave maximal yields and in the latter 5 to 15 mg PO_4^{--} per 25 ml (200 to 600 ppm).

Beijerinckia, on the other hand, was far more tolerant of extremely high phosphate levels than Azotobacter. The growth of Azotobacter was entirely checked at phosphate levels above 40 mg PO_4^{--} per 25 ml (1,600 ppm), but that of Beijerinckia was checked only at 580 mg PO_4^{--} per 25 ml (23,200 ppm).

The molybdenum requirements of Azotobacteriaceae form a very intriguing problem, because this element is essential for biological nitrogen fixation [6 7 17]. It was found that in spite of their high iron-concretion content, lateritic soils are molybdenum poor but certainly not molybdenum deficient. The experiments showed that the molybdenum concentration for half-maximal nitrogen fixation by Beijerinckia was about ten times higher than that of *Azotobacter vinelandii*, although the Beijerinckia strains were grown on glucose, containing traces of molybdenum as contamination, while the Azotobacter strain was grown on ethanol freed by redistillation from traces of molybdenum. The molybdenum requirement of Beijerinckia was, however, rather strain variable. Maximal and half-maximal nitrogen fixation in the *Beijerinckia indica* str. Hawaii 3/1 and str. Tanganyika 4/1 were attained with 0.2 and 0.08 ppm, and with 0.034 and 0.004 ppm of molybdenum,

respectively. The results obtained with the Beijerinckia strain from the Tanganyikan soil closely resembled those reported by Jensen [19] for the *Beijerinckia lacticogenes* [21] type strain.

In the Beijerinckia strains vanadium was unable to replace molybdenum in nitrogen fixation, confirming an earlier observation of Jensen [18]. Tests upon twenty-five other Beijerinckia strains gave similar results. The fact that for Beijerinckia – in contrast to Azotobacter – vanadium cannot be substituted for molybdenum in nitrogen fixation seemed to be an outstanding characteristic of this genus. However, by screening a great number of Azotobacter strains belonging to different species, it was discovered that vanadium could not replace molybdenum in any of the *Azotobacter agile* strains tested and nor again in some of the *Azotobacter chroococcum* strains. Thus, the general opinion that vanadium may be substituted for molybdenum in nitrogen fixation by Azotobacter does not hold for all strains.

Copper and zinc at a concentration of 1 ppm increased the amount of fixed nitrogen in the *Beijerinckia indica* str. Hawaii 3/1 by 24 and 12 per cent respectively. Mulder [28] observed that *Azotobacter chroococcum* needs copper for the development of the characteristic dark colonies of this species (tyrosinase activity), but he found no effect on growth and nitrogen fixation.

SUMMARY

The mineral nutrition of Beijerinckia was studied in relation to the mineral composition of lateritic soils of the tropics, which form its preferential habitat.

Consistent with the extremely low level of calcium in lateritic soils, Beijerinckia had – in contrast to most Azotobacter strains – no requirement for calcium. In addition, Beijerinckia was adapted to the very low pH values sometimes found in lateritic soils, for it can grow and actively fix nitrogen up to very acid reaction; some strains even showed optimal nitrogen fixation at pH 3.5 (Becking [5]).

In agreement with the very high iron levels occurring in lateritic soils, Beijerinckia was found to have a higher requirement for iron than Azotobacter, and in contrast to Azotobacter it had a remarkable resistance to extremely high iron levels even at a rather low pH.

The elements aluminium and titanium often present in large quantities in lateritic soils proved to be neither essential nor stimulatory for nitrogen fixation. However, Beijerinckia was far more tolerant than Azotobacter of high levels of these elements. Only at high Al-concentrations of 80 ppm

and initial pH 5.0 (final pH 3.0 to 3.2) was there a reduction in nitrogen fixation in some strains, but not in the very acid-tolerant *Beijerinckia mobile* str. 1.

Magnesium was found to be an essential element for growth in Beijerinckia. Jensen [19] observed a far lower magnesium requirement in Beijerinckia than in Azotobacter, an observation which tallies with the low magnesium status of most lateritic soils. In the present study, however, a Beijerinckia strain isolated from magnesium-rich latosol (derived from a Mg-rich parent material) was found to have a magnesium requirement of the same order as that of Azotobacter.

Manganese as trace element is apparently not essential for the growth of Beijerinckia, nor for an *Azotobacter chroococcum* strain from a temperate (Delft, Holland) soil. However, the nitrogen fixation of an *Azotobacter chroococcum* strain isolated from a manganese-rich tropical (Ngawi, C. Java) soil was considerably increased by the presence of this element. Further, Beijerinckia was shown to be very resistant to high manganese levels, indicating an adaptation to lateritic soils which are often manganese rich. Only the high manganese concentration of 400 ppm at pH 5.0 (final pH 3.0–3.2) reduced the nitrogen fixation to about 50 per cent.

Lateritic soils are known to be very low in available phosphate, owing to fixation by the iron complexes which are abundantly present in them. Correlated with this, Beijerinckia showed nitrogen fixation at far lower phosphate levels than *Azotobacter chroococcum* under identical conditions. On the other hand, Beijerinckia was far more resistant to high phosphate concentrations than Azotobacter.

In consequence of their high content of iron concretions, lateritic soils also are rather poor in molybdenum, although they are definitely not molybdenum deficient. The molybdenum concentration for half-maximal nitrogen fixation by Beijerinckia was found to be about ten times that required by *Azotobacter vinelandii*. The molybdenum requirement for maximal nitrogen fixation was, however, approximately the same in both species. Vanadium was found to be unable to replace molybdenum in the nitrogen fixation of Beijerinckia, but the general opinion that vanadium is always able to replace molybdenum in the nitrogen fixation of strains of the genus Azotobacter was shown not to be true for all of them. Vanadium could not replace molybdenum in the nitrogen fixation of all *Azotobacter agile* strains tested nor in some *Azotobacter chroococcum* strains.

One Beijerinckia strain tested showed a definite demand for copper and zinc for maximal nitrogen fixation.

In the context of these results, it is worth while emphasizing that the common belief in the beneficial effect of lime on symbiotic nitrogen fixation and on the presence of Azotobacter in soils does not hold for tropical soils. Apart from the fact that liming causes deterioration of the good soil structure of latosols and has a deleterious effect on the availability of minerals in these soils [25], Beijerinckia (1) requires no calcium for growth and nitrogen fixation, (2) is rather indifferent to low pH or has a very low pH optimum (pH 3.5) for growth and nitrogen fixation, and (3) calcium carbonate even inhibits its

development. Very recently, Norris [33 34 35] has drawn attention to the same facts with regard to the Rhizobium of the cow-pea type from tropical soils, which is able to fix nitrogen symbiotically with tropical legumes in very acid tropical soils and also showed no calcium requirement for growth.

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EFFECT OF VOLATILE CONSTITUENTS OF *SCAPTOCORIS TALPA* CHAMP. ON THE GROWTH OF SOIL FUNGI

by M. I. TIMONIN *

INTRODUCTION

Banana wilt, commonly known as Panama disease, caused by *Fusarium oxysporum* f. *cubense* (E.F.S.) Syn. and Hans., is a limiting factor in the production of Gros Michel bananas in Honduras. Many acres of productive land are abandoned annually due to this disease. On some of the abandoned araeas there are some banana plants remaining, the so-called "islands", which are still in production. During an examination of the profile of the root system of a banana plant, *Musa sapientum* L. variety Gros Michel, growing in such an island, it was noticed that the roots were heavily infested with hemipterous insects. Some of these insects were firmly attached by their rostrums to the fleshy and fibrous adventitious roots (Plate 1-A) and were found at a depth from 4 to 44 inches.

The insects (Plate 1-B) were identified as *Scaptocoris talpa* Champ. which belongs to the family Cydnidae of the suborder Heteroptera[15]. Adults and nymphs are provided with scent glands which emit a very repugnant odor. If the insects are present in the soil on excavation one can detect them by their odor.

It is an established fact that certain insects are vectors of human, animal, and plant diseases. However, it is also true that certain insects are able to produce a specific substance(s) which demonstrate antibacterial and antifungal properties *in vitro*.

Excellent reviews of the literature on production of antibacterial substances by insects and arachnids have been made by Perry [12],

* Vining C. Dunlap Laboratories, Tela Railroad Company, La Lima, Honduras (A subsidiary of United Fruit Co.). Present addres: Dept. of Forestry, University Sub Post Office, Saskatoon, Sask. Canada.

and later by Pavan [11]. Pavan [10] isolated an "antibiotic" from an Argentine ant which he named, after the generic name of the formicid, Iridomyrmecin. According to Pavan, Iridomyrmecin is active against Gram-negative, Gram-positive, an acid-fast bacteria and also demonstrates insecticidal properties. Alexander and Burton [1] and later, Locanti and Roth [8], isolated from the odorous secretion of *Tribolium castaneum* (Herbst) two quinones: 2-ethyl-1, 4-benzoquinone (80–90%) and 2-methyl-1,4-benzaquinone (10–20%). DeCoursey *et al.*[2] demonstrated that aqueous extracts of *T. castaneum* have a strong antibacterial activity *in vitro* against a wide spectrum of bacteria. However, these extracts showed no activity against 6 fungi tested.

Recently Fieser and Ardao [5] obtained an antibiotic from a South American arachnid (family Gonyleptidae) which was isolated in crystalline form and identified as a mixture of three quinones, the major constituent being 2,3-dimethyl-1,4-quinone. The authors named this compound "gonyleptidine". Gonyleptidine is a volatile substance and according to Estable *et al.*[3], has a bacteriostatic effect on Gram-positive as well as Gram-negative bacteria *in vitro*. Koidsumi [7] reported that cuticular lipids of the silk worm (*Bombyx mori* L.) and rice-stem borer (*Chilo simplex* Butl.) larvae contain antifungal matter, identified as caproic or caprylic acid.

As far as the author is aware, nothing has been reported in the literature on the relationship of *S. talpa* either to Panama disease or to the soil microflora, and the determination of this relationship was the object of the present investigation.

EXPERIMENTAL

Methods

Sufficient numbers of *S. talpa* were excavated, stored in soil in a wooden box at the laboratory and used when needed.

Soil for experimental purposes, obtained from the same location, was classified as a loam containing 50% fine sand, 38% silt, and 12% clay. The pH was from 7.2 to 7.3.

The moisture content of the soil was determined (at 105°C) and 500 grams of moist soil were weighed out and deposited in each of 12 one-quart glass jars. The jars were covered with non-absorbent cotton between two layers of cheese cloth and secured with string. One series of jars was autoclaved for 3 hours at 15 pounds pressure; the second series was not sterilized. Both series received the following treatments.

1. Control
2. 10 ml of conidial suspension of *F. oxysporum* f. *cubense*
3. 50 insects (adults and nymphs of *S. talpa*)
4. Treatment 2 + 3
5. 75 insects (adults and nymphs of *S. talpa*)
6. Treatment 2 + 5

For inoculum *F. oxysporum* f. *cubense*, yellow race [14] was grown on peptone—glucose agar (pH was not adjusted) for 6 days and then a suspension of conidia was made in sterile distilled water. By plating this suspension on peptone—glucose medium it was found that one ml contained 1,357,000 spores. Therefore, each 500 g of soil received 13,570,000 spores, or 34,794 spores per gram of moisture-free soil. After the addition of fungus inoculum and or insects, the soil was thoroughly mixed by rotating and shaking the jars so that a homogenous distribution of inoculum and insects was obtained. The moisture content of the soil then was brought up to 22% (w/w) with sterile distilled water and maintained at this level throughout the experiment. The jars were incubated at a temperature from 29 to 31°.

The numbers of the various groups of micro-organisms were determined by plating and dilution methods. Soil extract agar (pH from 3.8 to 4.0) and Martin's [9] peptone glucose media were used for counts of fungi. Soil extract (pH 6.8 to 7.0) and nutrient agar were used for estimation of numbers of bacteria, and counts of actinomycetes were made on sodium asparaginate—glycerol agar. The populations of nitrifying and denitrifying bacteria were estimated in Winogradsky's and Giltay's liquid media respectively [6].

The various procedures for concentration and for assay of the activity of the antifungal matter emitted by *S. talpa* are given in the text.

External and internal microflora of S. talpa

To determine whether *S. talpa* is a carrier of *Fussarium oxysporum* f. *cubense*, six insects were washed in five changes of sterile tap water. Suitable dilutions of a sample of each wash water were prepared and poured in petri dishes with soil-extract agar. The plates were incubated at room temperature from 28 to 31°C) for 10 days and then examined. No fungus colonies developed from any of the five samples of wash water used. However, abundant colonies of bacteria developed.

To investigate the internal microbial population, the six washed insects were macerated under aseptic conditions. Suitable dilutions of the macerated matter were prepared and plated with soil extract agar. The plates were examined after 10 days of incubation at room temperature. Numerous bacterial colonies were developed from all dilutions except one, this being a petri dish in which in-

sects were macerated but which was poured with agar medium without dilution. This dish developed a profuse growth of a phycomycetous fungus, bacteria, and a few colonies of actinomycetes.

To insure the contact of *S. talpa* with the pathogen, the insects were introduced into soil heavily inoculated with *F. oxysporum* f. *cunbese* (152,000 spores/gram of moist soil) and after 6 days of incubation the insects were removed from the soil and their external and internal fungus floras were determined. In spite of the fact that soil particles visible to the naked eye were attached to the insects, no Fusarium colonies developed from any of the 5 samples of wash water or the macerated insects. However, numerous colonies of a phycomycetous fungus developed on the plate in which the insects were macerated and which was poured with soil-extracts agar without dilution. From the results obtained it may be concluded that *S. talpa* is not a carrier of *F. oxysporum* f. *cubense*, externally or internally.

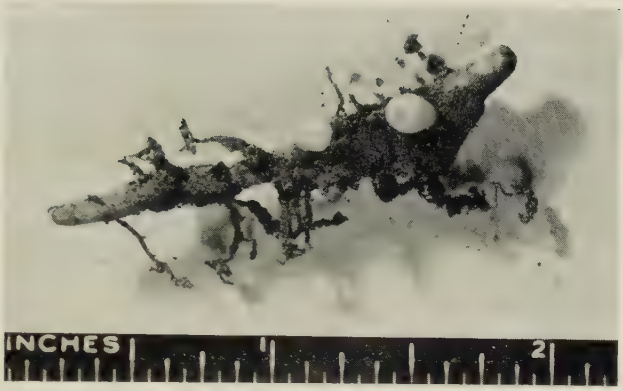
Effect of volatile matter of insect origin on the growth of certain fungi

To investigate the effect of the volatile matter emitted by *S. talpa* on the growth of fungi, plates with Czapek's medium were spot inoculated either with a bit of agar from a six day-old culture of *F. oxysporum* f. *cubense* or with a conidial suspension in sterile distilled water. After being inoculated, the plates were inverted and the bottom part of each plate was removed. Five insects were deposited on the lid of each plate and the bottom part replaced. Thus, the insects were not in contact with the inoculum but the volatile matter emitted by them filled the air space in the petri dish as indicated by the odor. The control plates were prepared in the same manner but were incubated without insects. All plates were incubated at room temperatures (from 29 to 31°).

After 40 to 48 hours of incubation the volatile matter emitted by the insects completely or partially inhibited the growth of the test fungi, whereas control cultures produced a normal growth.

Further investigation indicated that one insect per cubic inch of air was able to produce enough volatile matter to inhibit the growth of two races of *F. oxysporum* f. *cubense*, "yellow" and "non-yellow" [14], when the inoculum was a spore suspension in sterile distilled water. By sub-culturing the inocula after 48 hours of

A



B

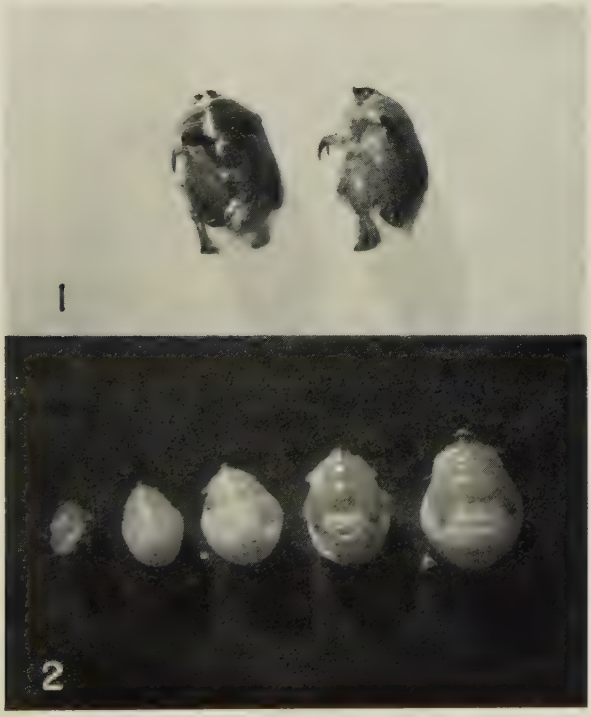


Plate 1. *Scaptocoris talpa.*
A - nymphs feeding on banana roots (magn $1^1/_2$).
B - adult insects (magn. ab. $3\times$).
Note the characteristic anterior and posterior legs.

Plate 2. Effect of odoriferous matter emitted by *S. talpa* on the growth of fungi. *Top:* one insect per cubic inch of air space; *bottom:* without insects (control).

incubation in the atmosphere of volatile matter emitted by *S. talpa*, it was found that one insect per cubic inch of air space produced a fungistatic effect on the inoculum, whereas two or three insects per cubic inch produced a fungicidal effect on the "yellow" as well as the "non-yellow" races. In all cases, however, the effect of volatile matter on the test organisms was only fungistatic when the inoculum used was a bit of agar from a six-day-old culture

TABLE 1

Effect of volatile matter emitted by various numbers of insects per cubic inch of air Effect of volatile matter emitted by various numbers of insects per cubic inch of air on the growth of two races of *F. oxysporum* f. *cubense* spot inoculated on the Czapek's solution agar and incubated at 29–31°.

Number of insects per cubic inch air space	*F. oxysporum f. cubense* races	Inoculum spores	Growth of subculture	Inoculum mycelium spores	Growth of subculture
1	Yellow	0	+ + ; + +	0	+ + ; + +
	Non-yellow	0	+ + ; + +	0	+ + ; + +
2	Yellow	0	— — ; — —	0	+ + ; + +
	Non-yellow	0	— — ; — —	0	+ + ; + +
3	Yellow	0	— — ; — —	0	+ + ; + +
	Non-yellow	0	— — ; — —	0	+ + ; + +

0 Indicates no visible growth after 48 hours incubation
+ + Indicates growth of subculture from test plate (fungistatic effect)
— — Indicates no growth of subculture from test plate (fungicidal effect)

(Table 1). The data presented in Table 2 indicate that one insect per cubic inch of air space inhibited the growth of the following fungi: *Aspergillus niger*, *A. terreus*, *Botrytis cinerea*, *Colletotrichum trifolii*, *Curvularia lunata*, *Fusarium graminearum*, *Fusarium* spp. (2 cultures isolated from soil), *Gliocladium* spp., *Penicillium verrucosum*, *Penicillium* spp., *Periconia circinata*, *Sclerotium rolfsii* (sclerotisa used for inoculum), *Stachybotrys atra*, and *Trichoderma viride*, (Plate 2), when the inoculum was made from a spore suspension.

It was noticed, however, that in the presence of insects, sclerotia of *S. rolfsii* germinated and produced some mycelial growth but later the mycelium collapsed and did not produce further growth. *Gliocladium deliquescens*, on the other hand, produced a thin layer of mycelial growth which after five days of incubation practically covered the plate.

TABLE 2

Effect of odoriferous matter of one insect (*S. talpa*) per one cubic inch of air space on the growth of fungi on Czapek's agar. (Incubation 48 hours at 28–31°C)

Test organisms	Colony diameter in mm	
	With insects	Without insects
Aspergillus niger	0	12
Aspergillus terreus	0	15
Botrytis cinerea	0	3
Colletotrichum trifolii	0	8
Curvularia lunata	0	18
F. oxysporum f. *cubense* isolates:		
"yellow"	0	20
"non-yellow"	0	32
"smooth"	0	18
"aerial"	0	25
Fusarium graminearum	0	30
Fusarium sp. (1)	0	8
Fusarium sp. (2)	0	12
Gliocladium deliquescens	8	60
Gliocladium sp.	±	10
Penicillium verrucosum	0	18
Penicillium sp.	0	15
Sclerotium rolfsii	±	8
Stachybotrys atra	0	10
Trichoderma viride	0	70

0 = no growth or conidia failed to germinate
± = conidia germinated but failed to produce mycelium

Effect of S. talpa *on the activity of* F. oxysporum *f.* cubense *and soil microflora in soil*

The results so far presented indicate the effect of odoriferous matter emitted by *S. talpa* on various fungi *in vitro*. Further experiments were outlined to determine the effect, if any, of these insects on the growth of *F. oxysporum* f. *cubense* and on the activity of soil micro-organisms in soil.

The jars with variously treated soil were incubated at room temperature (between 29 and 31°) for 6 days and the population density of different groups of micro-organisms was determined. The results sumarized in Table 3 indicate that the population of *F. oxysporum* f. *cubense* increased from 34,795 viable spores per gram of moisture-free soil to 383,000 and 93,000 in sterilized and unsterilized soil respectively. However, when *Fusarium* was added in combination with *S. talpa*, the population of *Fusarium* decreased from 34,794

TABLE 3

Microbial Population of the Soil as Affected by *Scaptocoris talpa*

Treatment *	Numbers of organisms per gram of oven-dry soil			
	Fungi in thousands	Fusarium in thousands	Bacteria in millions	Actinomycetes in millions
Sterilized soil				
1. Control	0	0	0	0
2. Fusarium	383	383	0	0
3. 50 insects	0	0	420	0
4. Fusarium + 50 insects	292	292	418	0
5. 75 insects	0	0	408	0
6. Fusarium + 75 insects	11	11	965	0
Unsterilized soil				
1. Control	8	0	22	1.5
2. Fusarium	111	93	21	1.2
3. 50 insects	0	0	57	1.3
4. Fusarium + 50 insects	54	38	63	2.1
5. 75 insects	0	0	57	1.6
6. Fusarium + 75 insects	35	12	78	1.3

* For outline of treatments see explanation in *Methods*

0 = No colonies developed on 1 : 10,000 dilution

spores per gram of soil to 11,000 in sterilized soil and to 12,000 in unsterilized soil. In the presence of *S. talpa* the total count of fungi also decreased from 111,000 to 35,000 per gram of moisture-free soil. The fungus flora in the insect-treated soil was composed mainly of phycomycetous fungi of which *Cunninghamella* was predominant. The population of bacteria in the insect-treated sterilized soil did not show any definite response to the treatments, except in Treatment 6. In non-sterilized soil, however, there was a definite increase in density of bacterial population from 22 million per gram of moisture-free soil, in soil without insects, to 57 and 78 million per gram of soil in the soil containing 50 and 75 insects respectively, per 500 grams of soil. The data also indicate that insects did not carry actinomycetous flora (sterilized soil) and had no effect on total counts of actinomycetes in the unsterilized soil.

From the data presented in Table 4 it is apparent that insects introduced a considerable number of denitrifying bacteria into the sterilized soil. However, the insects had no effect on the population of the indigenous denitrifying flora in the unsterilized soil. They also had no effect on indigenous nitrifying flora. Protozoa were

TABEL 4

Effect of *Scaptocoris talpa on* denitrifying processes in the soil

Treatment	Dilution of one gram of soil				
	1/10	1/100	1/100	1/10,000	1/100,000
Sterilized soil					
1. Control	— —	— —	— —	— —	— —
2. Fusarium	— —	— —	— —	— —	— —
3. Insects (50)	* *	* *	* *	— —	— —
	+ +	+ +	+ +	+ +	+ +
4. Fus. + Ins. (50)	* *	* *	— —	— —	— —
	+ +	+ +	+ +	+ +	+ +
5. Insects (75)	* *	* *	* *	— —	— —
	+ +	+ +	+ +	+ +	+ +
6. Fus. + Ins. (75)	* *	* *	— —	— —	— —
	+ +	+ +	+ +	+ +	+ +
Unsterilized soil					
1. Control	* *	* *	* *	* *	— —
	+ +	+ +	+ +	+ +	— —
2. Fusarium	* *	* *	* *	* *	— —
	+ +	+ +	+ +	+ +	+ —
3. Insects (50)	* *	* *	* *	* *	— —
	+ +	+ +	+ +	+ +	+ +
4. Fus. + Ins. (50)	* *	* *	* *	* —	— —
	+ +	+ +	+ +	+ +	+ +
5. Insects (75)	* *	* *	* *	* *	— —
	+ +	+ +	+ +	+ +	+ +
6. Fus. + Ins. (75)	* *	* *	* *	* *	* *
	+ +	+ +	+ +	+ +	+ +

\+ Indicates process of denitritification
* Indicates true denitrification with evolution of N gas
— Indicates no activity

observed in the control samples of unsterilized soil only. Amoebae were not observed.

Condensation of volatile matter emitted by S. talpa

By passing dry air or nitrogen gas into a flask containing between 300 and 400 insects and then through a trap flask immersed in dry ice and under reduced pressure (from 4 to 8 mm) it was possible to condense the volatile matter into an amorphous, whitish substance with a strong insect odor. On melting, this became an opalescent liquid containing a few oil like drops.

The volatile fraction of this liquid was assayed against two races of *F. oxysporum* f. *cubense* and against *Gliocladium deliquescens* and *Cunninghamella elegans*. The assay was performed by saturating

one "antibiotic assay disc" * with the liquid and placing the disc on the lid of the inverted standard 10-cm laboratory petri dish. Prior to this the petri dishes had been poured with Czapek's solution agar and, when cooled, spot inoculated with conidial suspensions of the test organisms. The petri dishes were then sealed with plasticine clay. Thus the assay disc was not in contact with the inoculum, but the volatile condensate emanated from the disc filled the air space of the petri dish. After 2 days of incubation at 29 to 30° the plates were examined and it was found that the growth of all organisms except *Cunninghamella* was inhibited. This indicates that the volatile matter emitted by insects can be condensed by the procedure outlined above.

Effect of vapors of volatile aliphatic monosabic acids on the growth of fungi

Koidsumi in his recent paper [7] reported that the cuticular lipids of the silk worm (*Bombix mori* L.) and rice-stem-borer (*Chilo simplex* Butl.) larvae contained antifungal matter. The antifungal matter, according to him, was identified as a caproic or caprylic acid. To compare the antifungal activity, if any, of the fumus of *n*-caproic and caprylic acids with the antifungal activities of condensed odoriferous volatile matter of *S. talpa*, all these substances were assayed against two isolates and two strains of *F. oxysporum* f. *cubense* and *Aspergillus niger*. The method for the assay was the same as for the assay of the condensed volatile matter emitted by *S. talpa*.

The results obtained indicate that fumes of *n*-caproic acid inhibit the growth of two isolates and "yellow race" of *F. oxysporum* f. *cubense* but fail to inhibit the growth of "non-yellow" race and *A. niger*. The fumes of caprylic acid retarded but did not inhibit completely the growth of all fungi. The condensate of the volatile matter emitted by *S. talpa*, under similar conditions, inhibited the growth of all test fungi.

DISCUSSION

The fact that *S. talpa* was found feeding on the roots of a banana plant growing on an "island" in an area abandoned due to Panama disease [15] suggested two possibilities: it might be a carrier of *F.*

* Disc. No. 740E, Carl Schleicher and Schuell Company, New York, U.S.A.

oxysporum f. *cubense*, the causative organism of Panama disease, or it might safeguard the plants from Panama disease.

The results presented in this paper indicate that *S. talpa* is not a carrier of Panama disease, but rather an antagonist to *Fusarium oxysporum* f. *cubense*.

The antagonistic activity of *S. talpa* is dependent on its ability to secrete an odoriferous matter. The data presented indicate that this odoriferous matter not only inhibits the germination of conidia of various soil-inhabiting fungi *in vitro* but also significantly reduces the density of the population of *F. oxysporum* f. *cubense* in unamended soil. It is believed this is the first insect reported which is able to produce antifungal substance(s) in unamended soil.

With regard to the identity of this odoriferous matter it should be pointed out that its chemical composition was not determined. However, both the condensate of this odoriferous matter and the ethanolic extract of *S. talpa* failed to react with $Na_2S_2O_4$ or to produce a quinhydrone on the addition of hydroquinone [4]. The volatile matter proved to be fungicidal or fungistatic to four isolates of *F. oxysporum* f. *cubense* and fourteen other fungi. It also failed to produce a yellow color on condensation whereas the quinones of insect origin were characterized by yellow color [1 5 8] and were not active against fungi [2]. This would tend to indicate the possibility that the volatile odoriferous matter produced by *S. talpa* is different from that secreted by *T. castaneum* [18] or the South American arachnid [3 5].

Recently Roth *et al.*[13] isolated a chemical compound, 2-hexenal (trans.), from odoriferous matter secreted by the cockroach, *Eurycotis floridiana*. This compound and its derivatives, according to the authors, has some insecticidal and insect-repellent properties.

The results on antifungal activity of the fumes of caproic acid indicate that the odoriferous matter of insect origin is a stronger antifungal agent than the fumes of caprylic or caproic acids. Furthermore, the volatile odor of these acids, singly or in combination does not resemble that emitted by *S. talpa*.

SUMMARY

An hemipterous insect found feeding on the roots of banana plants, variety Gros Michel, was identified as *Scaptocoris talpa* Champ. Adults and nymphs of this insect are provided with scent glands. *In vitro* the volatile odoriferous

matter secreted by these insects proved to be fungistatic or fungicidal, depending on concentration, to 4 isolates of *Fusarium oxysporum* f. *cubenze* and 14 other soil-inhabiting fungi. The condensed odoriferous substance seems to be different from quinones of insect origin or the fumes of caproic and caprylic acids. Under laboratory conditions, during 6 days of incubation in soil, (75 insects/500 g of soil) reduced the population of *F. oxysporum* f. *cubense* from 34, 794 viable spores per gram of moisture free soil to 11,000 and 12,000 in sterilized und unsterilized soil, respectively.

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SOME DIFFERENCES BETWEEN THE SOILS OF *RHIZOPHORA* AND *AVICENNIA* MANGROVE SWAMPS IN SIERRA LEONE

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INTRODUCTION

The most commonly occurring mangroves along the coast of Sierra Leone are *Rhizophora racemosa, R. mangle, R. harrisonii* and *Avicennia nitida*. Avicennia appears to grow on land less waterlogged than does Rhizophora but in some areas there are mixed stands of both kinds of mangrove, usually in a state of stunted growth.

Under natural conditions the swamps are flooded twice daily with salt water and for the successful cultivation of rice the land must be empoldered. Empoldering results in dessication of the topsoil during the dry season and thus to radical changes in the soil characteristics.

It has been recognised for many years that land cleared from Rhizophora tends to develop soil conditions adverse to rice growth when empoldered whereas land under Avicennia can usually be cleared and empoldered with impunity.

The Rhizophora-bearing soils are sulphidic and highly fibrous in nature and one of the major effects of empoldering is the production of sulphate in quantities sufficient to result in soil conditions toxic to rice; this aspect of the subject has already been discussed by Tomlinson [10] and more recently by Hart [6]. It is now well established that production of sulphuric acid together with concomitant liberation of aluminium ions is the explanation of the increased acidity of dried, fibrous mud from Rhizophora swamps over that of dried, non-fibrous mud from Avicennia swamps.

The origin of tne fibrous material in Rhizophora-bearing muds and its relationship to the low pH values obtained when the muds are dried will form the subject matter of another paper. The investigation here reported was designed to examine in more detail the differences existing between the two kinds of mud with particular reference to their behaviour on empoldering.

MATERIALS AND METHODS

The experimental site was a coastal mangrove swamp at Wellington near Freetown. A narrow fringe of *Rhizophora racemosa* bordered the seashore and behind this the vegetation became a mixed stand of *R. racemosa*, *R. mangle*, *R. harrisonii* and *Avicennia nitida* with the *Avicennia* predominant towards the higher ground. The soil under the *Rhizophora* and the mixed mangrove consisted of a soft, silty deposit of from 3 to 10 cm thick overlying a compact, fibrous layer of black mud which extended for several feet. Under the pure *Avicennia* the mud was greyish-brown, non-fibrous and contained more clay.

Analyses: All forms of sulphur with the exception of monosulphide were measured ultimately as sulphate by Hesse's [7] modification of the method of Chesnin and Yien [4], using a perchloric acid oxidation to sulphate where necessary. Sulphide was determined iodimetrically using potassium hypochlorite as an absorbent for liberated hydrogen sulphide (Little [9]). Sulphate was extracted with a solution of sodium acetate — acetic acid buffered to pH 4.5 and the same extract was used for measuring phosphate using Truog's reagent. Truog's reagent was also used to determine total phphosorus after wet oxidation of material. pH values were measured electrometrically with a glass electrode and carbon, nitrogen, ammonium, nitrate, chloride and silica were all determined by routine methods. The C/N ratios were calculated from the uncorrected Walkley-Black [11] carbon figures using the factor of 1.33.

For waterlogged and anaerobic incubations the samples were kept under one inch of standing water and for the anaerobic conditions all air was displaced by nitrogen. Respirometric studies were made using the macrorespirometer designed by Birch and Friend [3].

RESULTS

The main properties of the two kinds of mud and the overlying silt are shown in Table 1. The fibrous mud had a higher pH value than did the non-fibrous although as will be shown later, it produced more acid conditions on drying. The total-sulphur content of the fibrous mud was approximately four times that of the non-fibrous and although this was due in some part to a higher sulphate content,

TABLE 1

Composition of muds from Rhizophora and Avicennia mangrove swamps (Results expressed as % on oven-dry basis)

	Recently deposited tidal alluvium	Fibrous mud under Rhizophora	Non-fibrous mud under Avicennia
pH	6.4	6.6	6.2
Moisture	53.6	60.0	51.2
Carbonates	nil	nil	nil
Organic C	4.6	11.9	5.5
Total N	0.35	0.44	0.39
C/N	17	36	19
NH_4-N	0.0013	0.0010	0.0008
NO_3-N	0.0001	0.0001	0.0002
Total S	0.2444	2.224	0.577
Monosulphide-S	0.0091	0.0401	0.0067
SO_4-S	0.0470	0.1667	0.1250
Total oxidisable S	0.1970	1.0573	0.4520
Total P	0.0750	0.1510	0.1290
Acid-extractable P	0.0013	0.0018	0.0010
Cl	6.40	2.52	1.85
SiO_2	42.6	55.0	55.6

the difference was mainly in oxidisable sulphur. The fibrous mud also contained more chloride than did the non-fibrous; the greatest concentration of chloride however, appeared in the alluvial deposit.

The differences in total-nitrogen and phosphorus contents are all accounted for by the organic N and P in the fibrous material of the Rhizophora mud. The fibrous mud contained twice as much organic matter as did the non-fibrous and a very large difference in C/N ratios was found, 36/1 for the fibrous as opposed to 19/1 for the non-fibrous. One would expect considerable immobilisation of inorganic nitrogen in the fibrous mud with the wide ratio and to investigate this the mud was incubated for fifteen days with and without addition of ammonium sulphate (added at a rate of 2 cwt/acre. The results are given in Table 2 and confirm that the nitrogen of ammonium fertilisers added at this level would be rendered unavailable within two weeks.

When reclaiming polders in Holland it was found that the marine deposits contained sufficient carbonates to neutralise any acids formed when the muds were oxidised (Harmsen *et al.*[5]). Unfortunately, neither the muds of the mangrove swamp nor the silty deposit contained any measurable quantity of carbonate.

TABLE 2

The immobilisation of nitrogen by fibrous mangrove-swamp soil
(Results on oven-dry basis)

Soil	ppm NH_4-N		ppm NO_3-N	
	Initial	After 15 days	Initial	After 15 days
Mud alone	12.5	14.0	0	1.6
Mud + $(NH_4)_2SO_4$ at 2cwt/acre 6"	32.0	10.2	0	2.2

In the next experiment the two kinds of mud were incubated under aerobic, anaerobic, and waterlogged conditions and changes in pH, sulphate, phosphate, nitrate, and ammonium were followed. The analytical results are presented in Table 3 and help to bring out the differences in behaviour of the two muds. Both muds became more acid on aerobic incubation but the effect was more marked with the fibrous mud. Waterlogged and anaerobic condition raised the pH values of both muds and it was the fibrous mud in both cases which reached the higher value. Under aerobic conditions there was a small release of phosphate in the fibrous mud only,

TABLE 3

Changes occurring when fibrous and non-fibrous muds are incubated under various conditions
(Results on oven-dry basis)

Conditions	Incubation at 32°C for 30 days					
	Aerobic		Waterlogged		Anaerobic	
Mud	Fibrous	Non-fibrous	Fibrous	Non-fibrous	Fibrous	Non-fibrous
pH Initial	6.6	6.2	6.6	6.2	6.6	6.2
pH Final	4.6	5.7	7.0	6.3	7.2	6.9
NH_4-N ppm Initial	10	8	10	8	10	8
NH_4-N ppm Final	16	21	19	9	18	18
NO_3-N ppm Initial	1	2	1	2	1	2
NO_3-N ppm Final	2	4	0	4	0	0
PO_4-P ppm Initial	18	10	18	10	18	10
PO_4-P ppm Final	28	9	45	54	38	70
SO_4-S ppm Initial	1667	1250	1667	1250	1667	1250
SO_4-S ppm Final	4160	1240	1528	1148	1440	1024
CO_2-C produced ppm	940	885				

but under the more reducing conditions both muds liberated phosphate – again particularly the fibrous mud.

With aerobic incubation the quantity of nitrogen mineralised and which appeared mostly as ammonium, was not large but as the C/N ratios were greatly in excess of 5, ammonium formation

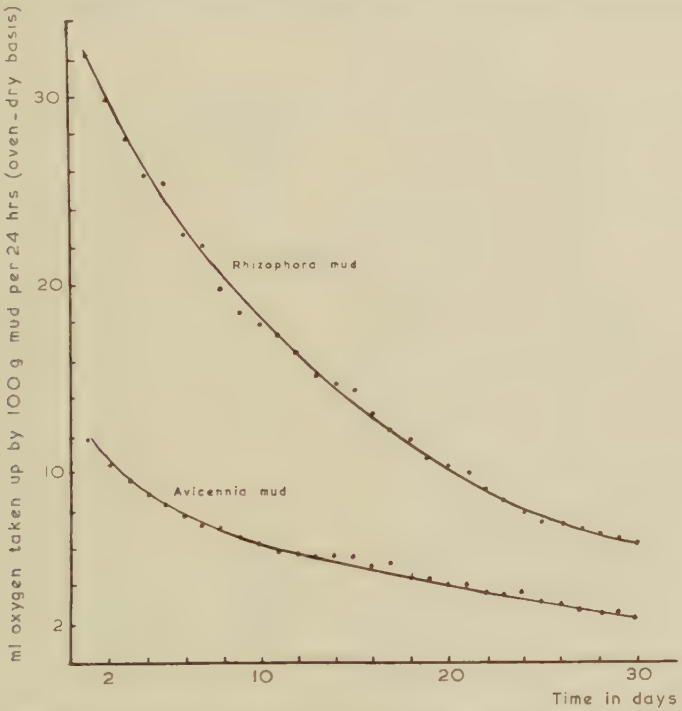

Fig. 1. Rates of oxygen uptake by muds from Rhizophora and Avicennia mangrove swamps.

would be retarded and furthermore, with such low C/S ratios (5 for the fibrous mud and 10 for the non-fibrous) one could expect little oxidation to nitrate (Barrow [1]).

The rates of oxygen uptake during aerobic incubation are shown in Figure 1 from which it would appear that the fibrous mud decomposed at a higher rate than did the non-fibrous. However, although the carbon oxidised in the fibrous mud (0.094 g in 30 days) was rather more than in the non-fibrous mud (0.0885 g), the main difference in oxygen uptake was due to sulphur oxidation. Thus in the fibrous mud the 0.094 g of carbon oxidised is equivalent to an

oxygen uptake of 175 ml and as can be seen in Table 3, 0.2493 g of sulphur were oxidised thus utilising a further 254 ml of oxygen, making a total of 429 ml of oxygen used to oxidise carbon and sulphur. The measured oxygen uptake converted to NTP was 438 ml. The oxidation of sulphur was not involved in the decomposition of the non-fibrous mud, the measured oxygen uptake of which

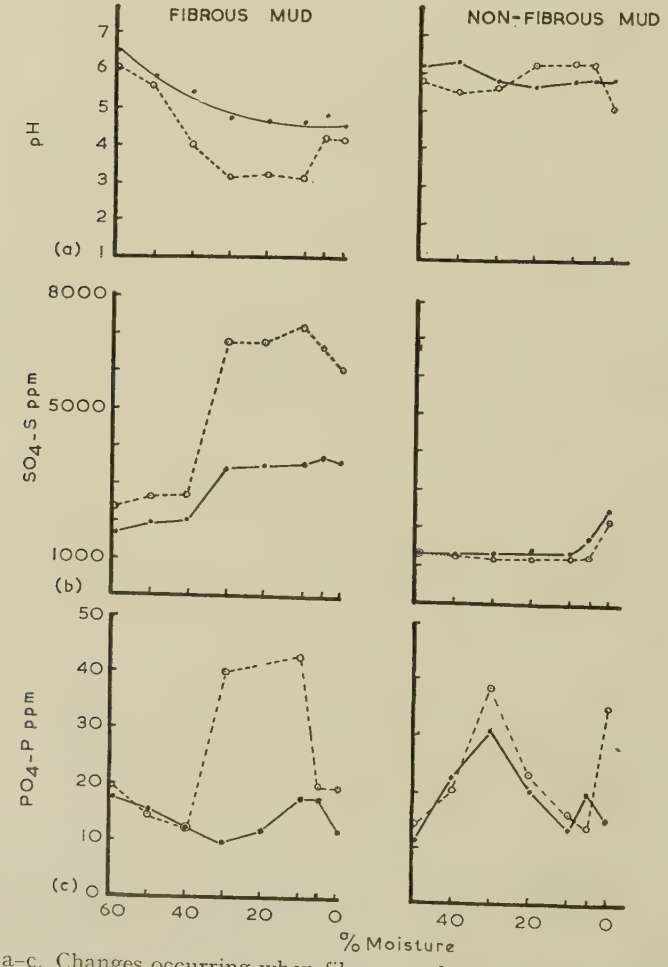

Fig. 2, a–c. Changes occurring when fibrous and non-fibrous muds are dried to various moisture contents (continuous lines) and the trend of such changes on incubation at any particular moisture content (broken lines). All points plotted on oven-dry basis.

was 170 ml at NTP and the oxidation of the 0.0885 g of carbon would account for 165 ml of this.

The investigation into the differences existing between the two kinds of mud was instigated by the fact that fibrous mud went strongly acid when dried whereas non-fibrous mud did not. The final and main experiment therefore was designed to examine in

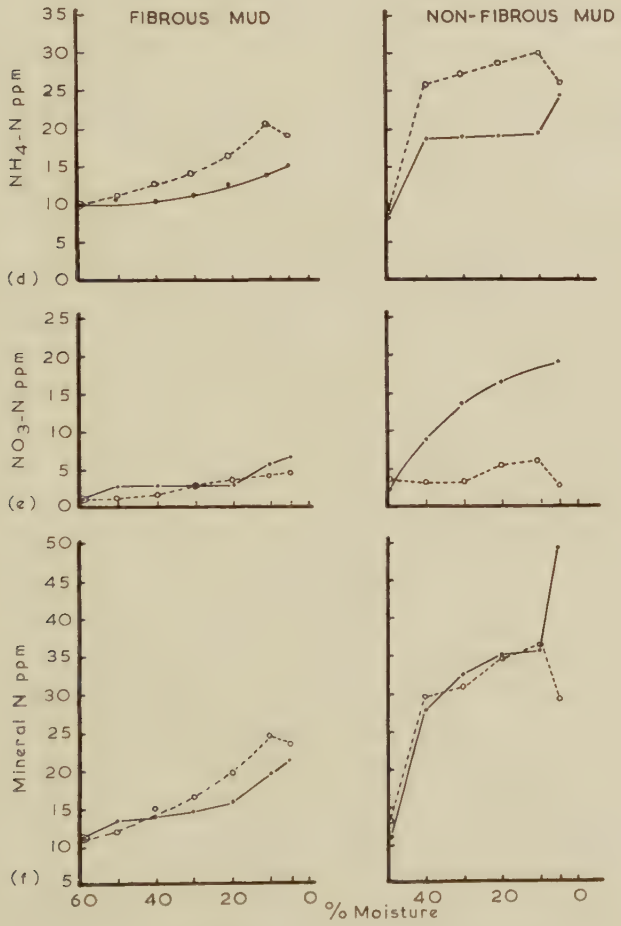

Fig. 2, d–f. See caption to Fig. 2, a–c.

more detail what changes occur when the muds are dried. Samples of mud were allowed to air-dry for different lengths of time so as to give muds with moisture contents varying from 5 per cent to field moisture at low tide; the latter was 60 per cent for the fibrous mud and 50 per cent for the non-fibrous ,the difference being due to the water-holding capacity of the fibre. Air-dry mud can be considered as that containing 5 per cent moisture and samples containing 0 per cent moisture were obtained by oven-drying at 110°C. The muds were then re-analysed and duplicate samples incubated in the respirometer for fifteen days. After incubation the muds were again analysed. The results are presented diagrammatically in Figure 2 (a–f) and show the changes which occur as the muds are continuously dried together with the trend of such changes if the drying should cease at any time.

As previously mentioned the most pronounced change which occurs on drying the fibrous mud is a drop in its pH value .In the mud here examined the decrease in pH was less drastic than in the mud described by Hart [6], but a sudden drop in the value took place at the same moisture content, that is at from 40 to 30 per cent. On incubation at moisture contents of from 40 to 10 per cent the pH decreased much more but at the low moisture contents of below 10 per cent no further change occurred with incubation. No significant change in pH occurred with the non-fibrous mud.

The sulphate changes in the fibrous mud followed those of the pH values, that is, a sudden production of sulphate took place when from 40 to 30 per cent moisture content was reached and which then remained fairly constant in amount. Similarly on storing, even more sulphate was formed. It can be seen from Table 1 that the fibrous mud contained 400 ppm sulphide sulphur and the sulphate sulphur formed during the initial drying from 60 to 40 per cent moisture did in fact amount to 400 ppm. This suggests that the sulphate first produced is by oxidation of the mono-sulphide present, microbiological oxidation of other sulphur compounds not occurring to any great extent until 40 per cent moisture is reached, a result in good agreement with Hart's [6] experiments on sulphur-oxidising bacteria. No change in the sulphate content of the non-fibrous mud was observed except for a small increase when drying to 0 per cent moisture in the oven. This confirms the corresponding drop in pH value at this point and is due to chemical oxidation of sulphur compounds.

The acid-soluble phosphate in the fibrous mud did not change on merely drying but on incubating it at the stage where the pH drops, a large increase in phosphate was obtained. This is almost certainly due to iron and aluminium phosphates coming into solution at pH 3. Drying the non-fibrous mud caused changes in acid soluble phosphate which, until further study has been made, cannot be adequately explained.

A steady increase in ammonium nitrogen took place as the fibrous mud dried and a further increase was apparent during incubation. Storing at a moisture content of 10 per cent resulted in the ammonium content being doubled. This larger increase at 10 per cent moisture is probably due to the fact that air-drying to this moisture took very much longer than drying to the higher moisture contents – even to 20 per cent. As found by Birch [2], the increase in ammonium nitrogen is a function of the length of time a soil is dry. The small change in ammonium on incubating below 10 per cent moisture was due to inactivity of micro-organisms. The non-fibrous mud when dried gave bigger increases in ammonium than did the fibrous mud in spite of the greater organic matter content of the latter. This is probably due to the acidity in the fibrous mud which would inhibit the ammonifyers.

It was expected that drying in the presence of lime would increase the amount of nitrogen mineralised in the fibrous mud and to test this fresh mud was mixed with 1% calcium carbonate and allowed to dry slowly until its moisture content was 5 per cent. It's pH value was then measured and the mud analysed for ammonium nitrogen. The results given in Table 4 demonstrate how mineralisation of nitrogen in the drying, fibrous mud is affected by the acidity produced.

TABLE 4

The effect upon nitrogen mineralisation of drying * fibrous mud with and without calcium carbonate
(Results on oven-dry basis)

Mud	pH	NH_4-N ppm
Fresh mud	6.7	10
Dried mud	3.1	15
Mud dried with $CaCO_3$ at 1%	6.1	736

* The mud was dried in air from 60% moisture to 5% over a period of several days.

GENERAL DISCUSSION

The nature of the muds in a coastal mangrove swamp appears to depend very largely upon the kind of mangrove they support although certain basic differences such as elevation and drainage, must originally decide which type of mangrove will become predominant. The differences between the two kinds of mud as found in undisturbed swamps of Rhizophora and Avicennia have been discussed already, the main points are that the fibrous mud associated with Rhizophora has a higher pH value, a higher content of oxidisable sulphur, nitrogen, and phosphorus, a higher C/N ratio and a larger water-holding capacity. The bulk of undecomposed organic matter in the fibrous mud is much greater than in the non-fibrous and as will be discussed in another paper, it is considered that the Rhizophora mangrove contributes more organic debris to the soil than does Avicennia.

The figures in Table 1 show that the fibrous mud contained over six times as much monosulphide as did the non-fibrous mud. As pointed out by Harmsen (Priv. comm.) the accumulation of sulphide sulphur in a Rhizophora swamp will depend mainly on the amount of organic matter desintegrated and which will have been oxidised with oxygen from the sulphate in sea water. Under the more aerobic conditions in muds under Avicennia, oxidation of organic matter will be performed partly by free atmospheric oxygen and thus muds of Rhizophora swamps will accumulate more oxidisable sulphur than those of Avicennia swamps.

Owing to the high C/N ratio of the fibrous mud and the consequent immobilisation of inorganic nitrogen (Table 2), land reclaimed from Rhizophora swamps should give a pronounced response to nitrogen fertilisers. The supply of nitrogen may well be a limiting factor in the fertilization of rice grown on such soils. At Calabar in Nigeria, an exploratory application of sulphate of ammonia at only 1 cwt/acre to an ex-Rhizophora swamp increased the yield of rice by 116 per cent (Otudor, Priv. comm.).

The differences in behaviour of the two muds when incubated under aerobic, anaerobic, and waterlogged conditions are summarised in Table 3 and have already been discussed. The greatest difference between the muds however, is in their behaviour when dried. It is, therefore, also the most important difference as rec-

lamation of the mangrove swamps will amongst other things involve the drying of the mud.

The production of strongly acid conditions in the fibrous mud when it is slowly dried affects all other changes; thus mineralisation of nitrogen is inhibited, iron and aluminium ions are released and acid-soluble phosphate increases. It has been shown that if for any reason the moisture content of the fibrous mud remained between 40 and 10 per cent for any length of time then the mud became even more acid. In practice this would almost certainly happen as a long time would be needed to dry the mud from 40 to 10 per cent and an occasional late shower of rain could easily occur.

Another important difference between the two kinds of mud is in the rate at which organic matter is oxidised when the dried muds are re-wetted and this has been described and discussed in a previous paper (Hesse [8]).

SUMMARY

The differences existing between a fibrous mud from a *Rhizophora* mangrove swamp and a non-fibrous mud from a nearby *Avicennia* swamp have been examined.

The differences found between the muds are due largely to the kind of mangrove they support.

Compared to the Avicennia swamp mud, that from the Rhizophora swamp had a higher pH value and a higher content of oxidisable sulphur, nitrogen, phosphorus, and carbon. The behaviour of the muds on incubation has helped to explain why the mud under Rhizophora is considered as being more fertile than mud under Avicennia. An apparent high rate of decomposition of the Rhizophora swamp mud as compared to that of mud under Avicennia was due to high oxygen uptake during sulphur oxidation. Rhizophora-bearing mud had a very high C/N ratio and inorganic nitrogen added to it was immobilised. It is suggested that land reclaimed from Rhizophora would respond to nitrogen fertilization. The most important difference between the muds was in their behaviour on drying. The intense acidity produced by sulphur oxidation in the fibrous mud affected all other changes, chemical and microbiological. It was found that if the moisture content of the fibrous mud remained at any point between 40 and 10 per cent for any length of time, then the mud became more acid than if continuously dried.

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THE EFFECT OF AMMONIUM NITROGEN ON FIXATION OF ELEMENTAL NITROGEN IN *ALNUS* AND *MYRICA*

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The ability of nodulated plants of *Alnus* and *Myrica* and of certain other non-legumes to fix atmospheric nitrogen at a rate sufficing to sustain vigorous growth in rooting media free of combined nitrogen is well established in respect of plants growing in artificial media in the greenhouse [5 6], and there is no doubt that if a suitable arrangement could be devised it would be possible to grow an alder tree to maturity without access at any stage to external combined nitrogen. It cannot be assumed that equally extensive fixation is associated with plants of these genera in the field, since new factors may operate there. One such factor is the usual presence in soils of some level of available combined nitrogen, for it is well known that in other nitrogen-fixing organisms combined nitrogen is used preferentially over the elemental form, fixation being reduced or even completely suppressed. One method of gaining information on this aspect would be by means of long-term soil analyses on areas of land of differing fertility planted with say alder. Holmsgaard [9] has recently collected together the rather sparse data of this type available in respect of alder, substantial fixation being indicated in most instances. A second method is to investigate the effect of combined nitrogen on fixation in plants in pot culture in the greenhouse, the method employed in the present study.

A considerable literature exists on the effect of combined nitrogen on fixation in pot cultures of legumes. Earlier investigators depended on painstaking analyses of rooting media and the harvested plants for the determination of nitrogen gained by fixation and by uptake of the combined form respectively, but more recently the simpler

and more accurate isotopic method has become available and was used by Thornton [12], Allos and Bartholomew [1] and other workers. A fair statement of the position in legumes, as indicated by the literature, seems to be that in the presence of sufficient combined nitrogen for vigorous plant growth, fixation per plant is reduced but is usually still quite substantial, and the legume grows only partly at the expense of the supplied combined nitrogen. The reduction in fixation is due mainly to the tendency for the total nodule mass per plant to decrease in the presence of combined nitrogen, but in addition there is some evidence of a reduction in fixation per unit nodule mass.

For the nodule-bearing non-legumes, information is available concerning the effect of combined nitrogen on nodulation, though the experience of different workers has varied somewhat [7 10 11]. In Glasgow experiments [7 10] with Alnus and Myrica substantial nodule development has occurred in the presence of various levels of ammonium nitrogen. It may also be recorded that alder trees under varied field conditions in Western Scotland bear seemingly abundant nodules, though quantitative data are hard to obtain. Bond [3] provided a few isotopic data indicative of the persistence of appreciable fixation of nitrogen in the nodules of Alnus and Myrica plants supplied with substantial levels of combined nitrogen. In the present more extensive study N^{15} has again been used, though now in a different manner.

METHODS

The principle of the isotopic method used in this study is as follows. Ammonium nitrogen, suitably enriched with N^{15}, is supplied at different levels to nodulated plants over a period of some weeks, starting at an early stage in growth. After harvest the total nitrogen content of the plants is determined, and also the degree of enrichment in N^{15} shown by this plant nitrogen. It is then possible to calculate how much of the plant nitrogen has been obtained by uptake of the supplied combined nitrogen, and by difference to discover the amount of atmospheric nitrogen concurrently fixed by the plants.

Raising plants

Seed of *Alnus glutinosa* and *Myrica gale* collected locally in the field was sown in vermiculite (Alnus) or peat (Myrica) after cold storage for some weeks at +2°C. Seedlings were transplanted into water culture when one

or two leaves had emerged. Containers for culture consisted of 2-litre glazed earthenware jars covered by squares of black polythene sheet (Alnus) or waxed teak (Myrica) bored with holes for the plants. The culture solution was that of Crone (nitrogen-free formula [2]), used at full strength and natural pH (6.2) for Alnus, but initially at one-quarter strength and with pH lowered to 5.4 for Myrica. A minor element supplement was added to the solution. At this stage there were six plants per jar. The greenhouse employed was lit by daylight.

Inoculation

Inoculation was effected with the plants in the solution without combined nitrogen, except that a single, uniform addition of unlabelled ammonium sulphate was made, providing 5 mg nitrogen per litre of culture solution, in order to assist the very small plants at this early stage. A drop of inoculum prepared by grinding field nodules in water with a little sand added (10 g nodules with 100 ml water for Alnus, 6 g with Myrica) was applied to the root system of each plant, a further 2 ml of the inoculum being added to the culture solution of each jar. This procedure was repeated two days later. The plants in a small number of jars were left uninoculated; these remained without nodules, neither did they receive any deliberate addition of combined nitrogen except that noted above. These plants showed negligible growth, and when harvested at the same time as the nodulated plants they showed a nitrogen content of 0.3 mg (Alnus) or 0.1 mg (Myrica) per plant, confirming that no unsuspected sources of combined nitrogen were available to the plants of the present investigation.

Preparation and application of labelled ammonium-nitrogen

An appropriate quantity of ammonium nitrate with 34 atom per cent excess N^{15} in the ammonium radical was made alkaline and the ammonia distilled over into dilute sulphuric acid. After neutralization of the excess acid, ordinary ammonium sulphate was added in amount calculated to reduce the excess N^{15} to approximately 1.5 atom per cent. The actual excess as determined by subsequent spectrometric assay proved to be 1.561 atom per cent. The ammonium nitrogen was brought into the form of a stock solution containing 20 mg nitrogen per ml.

The application of the labelled ammonium-nitrogen to the plants was commenced 7 weeks (Alnus) and 5 weeks (Myrica) after inoculation. The formation of nodules had naturally begun by this time but the plants were still very small, the height of the shoot being 2 to 3 cm, the dry weight (as determined on spare plants) of the order of 20 mg per plant, and the mean nitrogen content 0.3 mg (Alnus) or 0.8 mg (Myrica) per plant. Four levels of ammonium nitrogen were established, namely 0, 10, 50 and 100 mg nitrogen per litre of culture solution. Two jars each with six plants were set up at each nitrogen level for each species.

Subsequent treatment

The plants were grown for 10 to 12 weeks after commencement of treatment with the labelled nitrogen. After some two weeks the number of plants per jar was reduced from six to three in order to prevent eventual overcrowding, plants of average size being retained. Daily checks of the pH of the culture solution were made, and appropriate additions of NaOH made where necessary to restore pH to the original level of 6.2 (Alnus) or 5.4 (Myrica). The level of solution in the jars was restored daily by addition of distilled water; in the later stages of growth water uptake was considerable, with the result that those nodules situated on the upper parts of the roots were not constantly bathed by solution.

Three complete renewals of the culture solution in the jars were made during the period of treatment with labelled nitrogen, the strength of the Crone's solution being increased to one-third of full strength at the first renewal with Myrica. The levels of ammonium-nitrogen were restored to their original value at each renewal. In partial compensation for uptake by the plants, on a few occasions further additions of ammonium nitrogen were made during the periods between dates of renewal, the magnitude of these being decided on the basis of estimations of residual ammonium nitrogen in the culture solution. The harvest data (later) will provide further information on these aspects.

Harvest and analysis

At harvest the nodules were separated from each plant, counted, and their dry weight and also that of the combined root and shoot determined. 'Nodule' in this paper refers to the branched, clustered structure which in these non-legumes eventually arises from an original infection point. The dry matter from the three plants of each jar, including the nodules, was then combined and milled. Triplicate samples of this were taken for Kjeldahl analysis, allowing the calculation of total nitrogen per plant. The neutralised distillates from the replicate Kjeldahl analyses were combined, re-acidified and evaporated down to a small volume, a mass spectrometric assay being carried out on the concentrate from each jar of plants.

DATA OBTAINED

Alnus

Seedlings from seed sown 14th March were transferred to water culture on 18th April and first inoculated two days later. Treatment with labelled nitrogen was commenced 8th June and continued for some ten weeks, harvest being on 17th August. Six plants, in two jars, were available for harvest at each level of ammonium nitrogen.

All plants were of healthy appearance, as indicated by Plate 1A.

Plate 1.

A. Plants of *Alnus glutinosa* shortly prior to harvest, initially supplied with (left to right) 0, 10, 50 and 100 mg ammonium nitrogen per litre of culture solution. (× 1/11).

B. Plants of *Myrica gale* shortly prior to harvest, initially supplied with (left to right) 0, 10, 50 and 100 mg ammonium nitrogen per litre of culture solution. (× 1/11).

TABLE 1

Growth and dry weight data for *Alnus*							
(1) NH_4-N added per litre of culture solution, (mg)	(2) Mean height of shoot (cm)	(3) Mean number of leaves per plant	(4) Mean number of side shoots per plant	(5) Mean number of nodules per plant	(6) * Mean dry weight of nodules per plant (mg)	(7) * Mean dry weight of whole plant (mg)	(8) * Mean value for nodule weight as percentage of whole plant weight
0	34	19	2	64	144	3,123	4.6
10	52	38	7	69	243	6,677	3.6
50	52	62	10	32	230	9,257	2.5
100	56	62	11	25	158	10,634	1.5

* Appropriate statistical treatment of the original data indicates that the differences between means necessary for significance at $P = 0.05$ are as follows: Column (5), 30; Column (6), 75 mg; Column (7), 2,024 mg; Column (8), 0.9 per cent.

Growth and dry weight data are given in Table 1, with statistical treatment appended. The figures for whole plant dry weight (column 7) and the further data given in columns (2), (3) and (4) show that the provision of ammonium nitrogen at initial levels of 10 mg and 50 mg per litre resulted in successive large increases in growth, confirming that nodule action alone was incapable of satisfying the full nitrogen requirements of the plants. The dry weight with 100 mg ammonium nitrogen initially provided is not, however, significantly greater than at the 50-mg level, and it is fair to conclude that probably at both these levels, and certainly at the higher one, the nitrogen requirements of the plants were fully satisfied under the prevailing growth conditions. In agreement with previous Glasgow experience with Alnus, the greater growth of plants receiving 10 mg or 50 mg combined nitrogen per litre was accompanied by increased absolute weight of nodules per plant (column 6). Taking into account the data for number of nodules (column 5) it is clear that nodules tended to be larger in the presence of ammonium nitrogen. The additional formation of nodule tissues did not, however, keep pace with the increase in plant growth, so that in the relative sense (column 8) nodule development was continuously depressed.

Mean nitrogen data are provided in Table 2, with statistical and other information appended. As already explained, separate assays of N^{15} content (column 4) were made on the plant nitrogen of the

TABLE 2

Nitrogen data for *Alnus* *

(1) NH_4-N added per litre of culture solution (mg) †	(2) Mean percentage total N in plant dry matter	(3) ** Mean total N per plant (mg)	(4) ** Mean excess N^{15}, in plant N, atom per cent	(5) ** Mean mg NH_4-N absorbed per plant	(6) ** Mean mg atmospheric N fixed per plant	(7) ** N fixed as percentage of total uptake of N by plant
0	2.01	63	−0.006	0	63	100
10	1.94	130	0.371	31	99	77
50	1.87	174	0.950	105	69	39
100	1.96	208	1.193	159	49	24

* It should be noted that at each level of ammonium nitrogen two independent values were available for columns (2) to (7), each referring to a jar of three plants. For simplicity only the means of these pairs of figures are shown in the Table. Calculations made from the mean figures may not yield exactly the same results as those based on the original, separate data.

** Differences between means necessary for significance at $P = 0.05$ are as follows: Column (3), 37 mg; Column (4), 0.172 atom per cent; Column (5), 12 mg; Column (6), 35 mg; Column (7), 6 per cent.

† The amount of ammonium nitrogen supplied per plant during the period of the experiment was approximately as follows:

10-mg series	37 mg
50-mg series	127 mg
100-mg series	247 mg.

two jars of plants at each ammonium level. Agreement within each pair of figures was close, the maximum deviation of a single result from the mean being 8 per cent of the latter. An example will indicate the method by which the values in columns (5), (6) and (7) were calculated. In one of the jars of plants at the 10-mg level of ammonium nitrogen, the excess N^{15} in the plant nitrogen was 0.383 atom per cent as compared with 1.561 atom per cent present in the supplied ammonium nitrogen (p. 349). The mean total nitrogen per plant in this jar being 123 mg it follows that the amount of ammonium nitrogen taken up during growth was $0.383/1.561 \times 123$, or 30 mg per plant. By difference it is learned that the fixation of atmospheric nitrogen was 93 mg per plant. It is important that the figure for total nitrogen per plant (column 3) used in the calculation should represent the nitrogen accumulated by the plants during the period of treatment with labelled nitrogen. This requirement is adequately satisfied, since as noted on p. 349 the nitrogen content

at the commencement of the N^{15} treatment was less than 1 mg per plant. The assay figure for the zero nitrogen plants is not significantly different from the normal value.

The data in column (5) together with information appended to the Table show that at the 10-mg and 50-mg levels a large proportion of the supplied ammonium nitrogen was eventually absorbed by the plants. The data in column (6) show that fixation per plant was significantly enhanced in the 10-mg plants as compared with those at zero nitrogen, but was depressed in the presence of larger amounts of ammonium nitrogen. Nitrogen fixed as a percentage of total uptake of nitrogen by the plants (column 7) fell continuously as ammonium nitrogen was supplied in greater amounts, but it is notable that at the 100-mg level about one quarter of the over-all uptake of nitrogen was still due to fixation, despite the presence of a considerable amount of unabsorbed ammonium nitrogen in the rooting medium.

Myrica

Seedlings from a sowing of 17th February were transplanted into water culture on 20th April and first inoculated two days later. Treatment with labelled nitrogen was commenced on 29th May and continued for some 11 weeks until harvest on 12th August. Six plants, in two jars, were again available for harvest at each level of ammonium nitrogen.

These plants were also of very healthy appearance. Examples are

TABLE 3

Growth and dry weight data for *Myrica*

(1) NH_4-N added per litre of culture solution (mg)	(2) Mean height of shoot (cm)	(3) Mean number of leaves per plant	(4) Mean number of side shoots per plant	(5) Mean number of nodules per plant	(6) * Mean dry weight of nodules per plant (mg)	(7) * Mean dry weight of whole plant (mg)	(8) * Mean value for nodule weight as percentage of whole plant weight
0	40	69	5	243	197	3,273	6.0
10	47	57	6	238	191	3,884	4.9
50	50	102	6	195	163	5,447	3.0
100	52	76	6	189	162	4,587	3.5

* Differences between means necessary for significance at $P = 0.05$ are as follows: Column (5), 98; Column (6), 90 mg; Column (7), 2,533 mg; Column (8), 1.5 per cent.

TABLE 4

Nitrogen data for *Myrica* *

(1) NH_4-N added per litre of culture solution (mg) †	(2) Mean percentage total N in plant dry matter	(3) ** Mean total N per plant (mg)	(4) ** Mean excess N^{15} in plant N, atom per cent	(5) ** Mean mg NH_4-N absorbed per plant	(6) ** Mean mg atmospheric N fixed per plant	(7) ** N fixed as percentage of total uptake of N by plant
0	2.59	84	0.004	0	84	100
10	2.64	101	0.496	32	69	68
50	2.80	150	0.867	82	69	45
100	2.62	121	1.041	81	40	33

* The footnote to Table 2 referring to the method of calculation also applies to the above Table.

** Differences between means necessary for significance at $P = 0.05$ are as follows: Column (3), 84 mg; Column (4), 0.299 atom per cent; Column (5), 38 mg; Column (6), 55 mg; Column (7), 11 per cent.

† The amount of ammonium nitrogen supplied per plant during the period of the experiment was approximately as follows:

10-mg series . . . . . 36 mg
50-mg series . . . . . 135 mg
100-mg series . . . . . 264 mg.

shown in Plate 1B, while growth and dry weight data are provided in Table 3. Compared with some earlier Glasgow studies on Myrica [10] growth was greatly improved, especially in respect of the plants in nitrogen-free solution, this being due chiefly to the use of diluted rather than full-strength Crone's solution [8]. There was, however, appreciable variation in size from plant to plant, with the result that the differences between means required for statistical significance are rather large. Thus although the various data suggest that the addition of ammonium nitrogen increased growth, actually the changes in dry weight (column 7) fail to attain significance. The beneficial effect, if any, is however clearly smaller than in Alnus. The data also suggest that the 50-mg level of ammonium nitrogen, together with such fixation as occurred (below), did completely satisfy the nitrogen requirement. A comparison of the data in Colums (5) and (6) of Table 3 suggests that, as in Alnus, the nodules which formed in the presence of ammonium nitrogen tended to be larger, but the differences between the various means do not attain statistical significance. The relative weight

of nodules formed (column 8) was significantly depressed by ammonium nitrogen.

The nitrogen data are provided in Table 4. Those which are directly dependent on absolute size of plant (columns 3, 5 and 6) show rather low statistical significance. It is at least probable that practically all the supplied ammonium nitrogen was eventually absorbed by the plants at the 10-mg level, with lower proportions absorbed at the higher levels than with Alnus. Also, fixation per plant almost certainly fell as ammonium nitrogen was increased. The figures for excess N^{15} in the plant nitrogen of duplicate jars, on the other hand, again agreed satisfactorily, the maximum deviation of a single result from the corresponding mean being 9 per cent of the latter, indicating that at a given level of combined nitrogen plant size is not an important factor governing the relative importance of uptake of nitrogen in the ammonium form against that in the elemental form. Correspondingly the data in column (7) have good significance, and show a significant fall in the proportion of nitrogen fixed for each increase in ammonium nitrogen supplied. However, in the 100-mg plants, fixation still accounted for a third of the total uptake of nitrogen.

DISCUSSION

It has to be borne in mind that it was not possible to maintain steady levels of ammonium nitrogen in the rooting medium; thus it is clear from the data furnished that in the 10-mg series the culture solution must have been at times almost devoid of combined nitrogen, especially in the later stages of the growth period. In the 50-mg series there was almost certainly some ammonium nitrogen present at all stages, while at the 100-mg level there was a considerable quantity. Obviously the mean ammonium levels during the growth period were substantially below those provided initially and at each occasion of replenishment, but the data indicate that with each species at least the highest level more than satisfied the requirements of the plants.

As shown, substantial nodule formation occurred in Alnus and Myrica in the presence of various levels of ammonium nitrogen in the rooting medium. As the ammonium level was increased, nodules eventually became fewer but larger, the weight per plant in the

presence of an excess of ammonium nitrogen being still of the same order as in zero nitrogen. It is true that in this particular study, in order to reduce the consumption of labelled nitrogen, the first application of ammonium nitrogen was delayed until nodule formation had actually started, but as already emphasised the plants were still extremely small, and it is obvious that practically all growth of plants and nodules occurred subsequently to the commencement of the ammonium treatment. These effects of ammonium nitrogen on nodulation in Alnus agree with previous experience [7] [10]. The new data on Myrica, though somewhat tentative, are to be preferred to earlier ones since plant growth was so much improved. A fuller study of the effect of combined nitrogen on nodulation in Myrica will be reported elsewhere.

The object of the present study was to find how the role of the nodules forming in the presence of combined nitrogen is affected as compared with those on plants in nitrogen-free solution. They are obviously of reduced importance to the plant, which now bases its nitrogen metabolism to an increasing extent on the supplied ammonium nitrogen. Moreover, especially in Alnus, the plant grows more strongly and acquires a higher nitrogen content than in nitrogen-free solution, so that the relative significance of a given contribution from the nodules is steadily diminished. The data have shown that the nodules continue to fix atmospheric nitrogen. Whether they fix it as efficiently as in nitrogen-free solution is indicated when, from data already provided, the mg nitrogen fixed per g nodule dry matter formed is calculated for each treatment. The results are as follows:

Plant	NH_4-N added per litre of culture solution, mg			
	0	10	50	100
Alnus . . .	438	410	315	310
Myrica . . .	429	366	414	247

The differences required for significance between these values are 101 for Alnus and 112 for Myrica. Thus there is evidence of a certain reduction in efficiency of fixation.

This reduction could conceivably be due to a curtailment of metabolic supplies to the nodules, *e.g.* of acceptor substances for the

fixed nitrogen, resulting from the more vigorous nitrogen metabolism of the plant itself. Another possible reason is that there is a partial substitution of ammonium nitrogen for free nitrogen at the nitrogen-fixing centres in the nodule. This is an aspect seldom discussed even in connection with legume nodules. As noted already, in nitrogen-fixing systems in general ammonium nitrogen is used preferentially over elemental nitrogen. There is no obvious reason for believing that this will not also be true of fixation in root nodules, and it is intended to test the matter by means of isotopic experiments with detached nodules or with homogenised nodules. The fact that fixation, as shown by the figures given above, is not greatly reduced suggests that the ammonium nitrogen in the rooting medium does not penetrate freely into the nodules; an opportunity of securing evidence on this point was missed by our failure to arrange for separate N^{15} assays of the nodule nitrogen. However it may be noted that evidence has been advanced previously [4] suggesting that at least direct uptake of solutes from the rooting medium by these nodules is unlikely to be extensive. For this and other reasons the circumstance that in the later stages of the present experiments some nodules were not constantly bathed by culture solution (p. 350) is not thought to be important.

The differences in the effect of ammonium nitrogen on Alnus and Myrica call for a few observations. The data show that the over-all growth of the plants was much more stimulated in Alnus; also in the latter there was at low levels of ammonium nitrogen a substantially increased weight of nodules per plant, while associated with this was a greater fixation per plant. These effects are obviously due to the Alnus nodules in nitrogen-free solution failing by a larger margin than those of Myrica to satisfy the plant's requirements for nitrogen and to permit maximum nodulation. This could be due to an intrinsic inferiority on the part of Alnus nodules. An alternative and more probable explanation is that the cultural conditions were in some unknown respect less optimal for Alnus nodule activity than for those of Myrica.

From the field aspect the most important conclusion warranted by the observations is that the presence of combined nitrogen in the soil, even in considerable quantity, is unlikely to prevent substantial fixation in such nodules as are present. Although fixation will then only account for part of the total nitrogen uptake

by the plant, its magnitude per plant may be of the same order as in a soil destitute of combined nitrogen.

The results obtained portray a state of affairs in these non-legume plants which is very similar to that revealed by previous workers in legumes. Thus Allos and Bartholomew [1] found that various legumes still fixed substantial amounts of elemental nitrogen despite the presence of high levels of ammonium nitrogen in the rooting medium, though on account of extensive uptake of the combined nitrogen the fixation represented only a rather low proportion of the total nitrogen accumulation. The basic similarity of the legume and non-legume nodules in their physiological aspects is thus further emphasised.

SUMMARY

1. A substantial formation of nodules occurred on plants of *Alnus glutinosa* and *Myrica gale* grown in water culture with different levels of ammonium nitrogen labelled with N^{15} present in the culture solution. The nodules tended to be fewer but larger than on plants in solution free of combined nitrogen.

2. The nodules continued to fix atmospheric nitrogen despite the presence of ammonium nitrogen in the rooting medium, though fixation per unit weight of nodule tissues formed was somewhat lower than in nitrogen-free solution. Among other possible reasons this could have been due to a substitution of ammonium nitrogen for elemental nitrogen at the nitrogen-fixing centres of the nodule, but evidently this does not occur to any great extent.

3. In Alnus but not in Myrica fixation per plant was considerably enhanced in the presence of a low level of ammonium nitrogen, owing to greater nodule development. At higher ammonium levels, in excess of the plants' requirements, fixation per plant was still of comparable order to that in nitrogen-free solution, but now only represented some 24 to 45 per cent of the total nitrogen accumulated by the plants.

4. The results suggest that under field conditions some fixation of atmospheric nitrogen will always be associated with nodules present.

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THE EFFECT OF OXYGEN CONCENTRATION ON THE DECOMPOSITION OF ORGANIC MATERIALS IN SOIL

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INTRODUCTION

Many organic materials, on decomposition in widely different soils, give approximately the same products. Under fully aerobic conditions decomposition is accompanied by respiration, synthesis of microbial material and rapid disappearance of simple organic compounds [5 8 9 16 21]. In the absence of oxygen, volatile fatty and other organic acids accumulate and synthesis of microbial material is suppressed [2 7 1 9]. The effect of oxygen concentration on the balance of these microbial processes taking place in soil, however, has not been studied to any great extent.

Oxygen required by the micro-organisms travels first through the soil atmosphere and then through the soil water to the microbial surfaces. Since oxygen diffuses 10,000 times faster through air than through water, there are always large variations in the oxygen concentration in the soil water and only small variations in oxygen partial pressures in the soil atmosphere. The oxygen concentration in the soil water on the surfaces of the micro-organisms, therefore, should be correlated with their activities. The object of the work was to devise techniques for measuring this oxygen concentration and to correlate it with the mechanism of organic matter decomposition.

THEORY

The rate of respiration m of some micro-organisms [10]; is known to be related to the concentration of oxygen, c, by the equation

$$m = \frac{nc}{c + K_M}$$

where n is the respiration rate when oxygen is not limiting and K_M is the Michaelis constant. If K_M is small compared with c an adequate approximation to the curve can be made by taking m constant and independent of oxygen concentration for $c > C_0$ and zero for $c \leqslant C_0$. Consider a sphere of soil saturated with water and containing a uniform distribution of micro-organisms. Suppose the oxygen concentration is c at distance r from the centre of the sphere, D is the diffusion constant of oxygen through the sphere and t is the time. Then if the above approximation is made for M, the rate of soil respiration expressed per unit of respiring volume,

$$\frac{\delta c}{\delta t} = \frac{D}{r}\frac{\delta^2(rc)}{\delta r^2} - M. \tag{1}$$

If $r = R_0$ when $c = C_0$ and the oxygen concentration on the surfaces of the crumbs is C_1 i.e. at $r = R$, then in the stationary state [4]

$$C_1 - C_0 = \frac{M(R^2 - R_0^2)}{6D}. \tag{2}$$

If V_1 = volume of the sphere having oxygen concentrations between C_0 and C_1 and having a respiration rate of M, and V_2 = total volume of the sphere, then

$$C_1 - C_0 = \frac{MR^2}{6D}\left[1 - \left(1 - \frac{V_1}{V_2}\right)^{\frac{2}{3}}\right]. \tag{3}$$

If K is the rate of respiration expressed per unit of total volume V_2, which is made up of the volume V_1 and also the volume not respiring, then

$$C_1 - C_0 = \frac{MR^2}{6D}\left[1 - \left(1 - \frac{K}{M}\right)^{\frac{2}{3}}\right] \tag{4}$$

$$\left(1 - \frac{K}{M}\right)^{\frac{2}{3}} \simeq 1 - \tfrac{2}{3}\frac{K}{M} \text{ for } \frac{K}{M} \text{ small}$$

so that

$$\frac{MR^2}{6D}\left(1 - \left(1 - \frac{K}{M}\right)^{\frac{2}{3}}\right) \simeq \frac{KR^2}{9D} \text{ for } \frac{K}{M} \text{ small.}$$

In approximating to the exact solution as defined in Eq. (4) for values $K/M < 0.6$ the error introduced is $< 10\%$.

If there is a sample of crumbs that can be divided into groups having mean radii, $R_1, R_2, \ldots, R_n$ and they occupy volumes $v_1, v_2, \ldots, v_n$ then

$$R^2 = \frac{1}{\Sigma_1^n v_n} [v_1 R_1^2 + v_2 R_2^2 + \ldots + v_n R_n^2]. \qquad (5)$$

EXPERIMENTAL

Soils

The soils used were all taken from the surface 0–25 cm.

Soil	Description
A	Permanent pasture; clay loam; C = 2.6%, N = 0.36% (by Kjeldahl) and pH = 7.8 at a soil: water ratio of 1 : 2 (v/v)
B	Garden soil; clay loam; C = 2.5%, N = 0.32%, pH = 7.8
C	Brown earth; loam; C = 3.2%, N = 0.34%, pH = 5.3
D	Heath; C = 15.6%, N = 1.10%, pH = 4.2
E	Garden soil used in some previous work [5 7 8]; sandy loam; C = 3.7%, N = 0.4%, pH = 6.5

Before use, all soils were air dried and sieved between the limits of 2 mm and of 4 mm. The above organic carbon (C), nitrogen, and pH data refer to these fractions and they are expressed on an oven-dry basis. Organic carbon data are expressed as "Walkley and Black values" and have been obtained without use of a recovery factor (Piper [15], p. 227).

Materials

The amino acids used were all in the DL-form except for glutamic acid which was used in its L-form. Casein was acid hydrolysed; its amino acid composition was assumed to be that described by Spector [18]; and its purity was checked by α-amino-N determinations. All volatile fatty acids were of the reagent grade except for acetic which was AnalaR. Their purity was checked by volatile fatty acid analyses. Before use all acid solutions were brought to pH 7.0 with NaOH.

α-Amino-N and volatile fatty acid

Both were estimated as in previous work [7], except that a slight modification was used in the method of estimating volatile fatty acids. A Pregle instead of a Markham apparatus was used for steam distillation and distillate was collected in 50-ml fractions until no more volatile fatty acid distilled over.

To test the recovery of volatile fatty acid, soil A (4 g) was shaken for 30 min with 200 μ-equivalents of each acid dissolved in 20 ml of water, then filtered and the filtrate analysed. Minimal recoveries of 95 per cent were obtained with formic acid and of 98 per cent with acetic, propionic, butyric and valeric acids.

Stimulated-soil and soil-percolation techniques

These techniques were similar to those previously described [8]. The electrolytic rocking percolator [6] was used in percolation experiments where the soil atmosphere was maintained at a constant oxygen partial pressure. The only modification made in the electrolytic rocking percolation technique was that the total gas pressure inside the percolator was maintained at 73 cm instead of 76 cm of mercury. In this way difficulties of securing percolator caps *etc.*, were eliminated.

Warburg manometry

This was standard [20]; temp. 25°C, amplitude 3 cm; 100 cycles /min.
In some experiments there was no shaking.

Gas mixtures

These were prepared by the displacement procedure of Umbreit, Burris and Stauffer [20], p. 72.

Soil-moisture content

About 1 g of soil, accurately weighed, was heated at 105°C for 24 h, in an open specimen bottle, transferred to a desiccator, allowed to cool, and reweighed. The moisture content was calculated as percentage loss of the original weight. All dry weights are expressed on this basis.

Density of soil crumbs

Soil crumbs (about 5 g) were added to a specific-gravity bottle, and the weight of crumbs determined by weighing before and after transfer. After the addition of 10 ml of distilled water, the bottle was placed in a desiccator and evacuated for 30 min. The bottle was then filled with distilled water and reweighed. The density of soil A was 2.40 $g \cdot cm^{-2}$.

Determination of the mean square of the radius of moist soil crumbs

Dry soil crumbs were divided into seven groups according to their radii. A series of different sized circles was prepared on a sheet of paper. Their

radii were of the same order of size as those of the crumbs. Each crumb was placed over each of the circles and the radius of the one most nearly covered was noted. In this way it was found that X_1 dry crumbs had a mean apparent radius of r_1, and X_2 dry crumbs had a radius of r_2 and so on. The volume occupied by the total sample of crumbs when they contained 59 per cent water (v/v) (as in both percolation and Warburg manometric experiments) was estimated from dry weight and density measurements. If this volume occupied by the crumbs when moist is V_t and if the ratio of moist volume/dry volume of the crumbs is w then

$$\tfrac{4}{3}\pi w[X_1 r_1^3 + X_2 r_2^3 + \ldots X_n r_n^3] = V_t. \qquad (7)$$

Substitution of the known values of X_i, r_i ($i = 1, \ldots, n$) and V_t in Eq. (7) gave w.

If the radius of the X_i crumbs when moist is R_i then

$$R_i^3 = w r_i^3 \qquad (i = 1, \ldots, n).$$

If the volume of X_i crumbs when moist is v_i then

$$v_i = X_i \tfrac{4}{3}\pi R_i^3 \qquad (i = 1, \ldots, n).$$

Substitution of R_i and v_i ($i = 1, \ldots, n$) in Eq. (5) gives the required mean square of the radius.

Effect of oxygen partial pressure on the respiration of soil crumbs in Warburg flasks

Soil crumbs, (1.5 g) were placed in Warburg flasks and were moistened by the slow and careful addition of 0.9 ml of water. Any air trapped in the crumbs was removed by placing the flasks in a desiccator and evacuating for 30 min. The oxygen partial pressure in the flasks was then altered using the method of Umbreit, Burris and Stauffer [20] p. 70, and respiration rates were measured with the flasks stationary, at a temp. of 25°C .In some experiments, alteration of the oxygen partial pressure was not made until after the moist crumbs had been allowed to stand overnight in air. Both methods gave the same results.

Comparison of the respiration rates of casein digest-stimulated soil–water mixtures with those of yeast–sterilised soil–water mixtures

Soil A was stimulated to casein digest decomposition by percolation of casein digest (7.5 mg of α-amino-N) dissolved in 100 ml of water through 10 g of soil A. The soil was removed from the percolators 72 h after the start of percolation, thoroughly mixed and transferred in 1-g lots to Warburg flasks. Each flask also contained 3 ml of water and the oxygen partial pressure in the flask was altered using the method of Umbreit, Burris and Stauffer [20], p. 70. Substrate, 0.3 ml of casein digest (250 μg of α-amino-N) was added from the side arm, the flasks were set shaking and manometric readings taken. Control experiments were carried out with soils that had been percolated with water instead of casein digest.

Soil crumbs (dry sterilised by heating overnight in an oven at 100°C) 1% glucose in 0.1 *M* phosphate buffer pH 7, and yeast suspended in the buffer, were added to Warburg flasks so that the weight of soil, the volume of water, and the respiration rate under an atmosphere of air, were identical with those of the stimulated soil—water mixtures described above. The rate of rocking and the temperature were also identical in the two sets of experiments. After the oxygen partial pressure in the Warburg flasks had been altered, manometric readings were taken.

Respiration rates were measured for 240 min and during this time they were all constant and independent of time.

RESULTS

Warburg manometric experiments showed that the effect of oxygen partial pressure on the respiration rates of soil crumbs could be expressed in the form of Eq. (4) as is shown in Fig. 1. During the experiment (about 300 min) the respiration rate at each oxygen partial pressure was independent of time and therefore showed that stationary state conditions were maintained. The

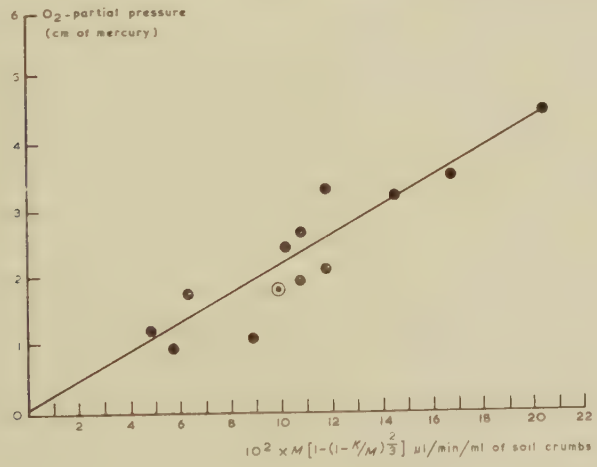

Fig. 1. Effect of oxygen partial pressure on the respiration of soil crumbs in Warburg flasks. Conditions were as described in Experimental Section. Crumbs were of soil A and when water-saturated they had a mean square of the radius (R^2) of 2.4×10^{-2} cm^2, and contained 59% water (v/v). Respiration rate under an oxygen partial pressure of 16.0 cm of mercury varied from experiment to experiment but was about 0.3 µl/min/ml of soil crumbs. The line fits Eq. (4) and the calculated best fit is $y = 21.5x + 0.045$. –⊙– is a point calculated from Fig. 2.

oxygen concentration, C_0, at which respiration is inhibited according to Eq. (4) is given by the intercept on the y-axis; it equals in Fig. 1 an oxygen partial pressure of 0.045 cm of mercury or an oxygen concentration in water of 0.7×10^{-6} M. The value for the diffusion constant D of oxygen through water-saturated soil crumbs calculated from the gradient of Fig. 1 was 0.82×10^{-5} cm^2sec^{-1} and that calculated by an entirely different method using Penman's [14] formula was $1. \times 10^{-5}$ cm^2sec^{-1}. The values obtained by the two methods in other experiments agreed within 25 per cent, which is satisfactory as there were almost certainly large errors in determining the mean square of the radius of the crumbs.

Some of the assumptions of Eq. (4) were justified by the results of following experiments. Evidence for the uniform distribution of

TABLE 1

Distribution of micro-organisms in soil

The oxygen uptakes obtained on shaking 1 g of the indicated fraction of soil A with either 3 ml of water or with 3 ml of casein digest solution containing 120 μg of α-amino-N. Experiments were carried out in Warburg flasks under an atmosphere of air for 180 min in absence of substrate, and for 275 min in presence of casein digest. Average S.E. is ± 5 (3)

Treatment	Fraction of soil (diam.)	Oxygen uptake (μl)
Water	2–4 mm	65
Water	< 2 mm	67
Casein digest . . .	2–4 mm	168
Casein digest . . .	< 2 mm	171

micro-organisms in soil crumbs was given by experiments which showed that the respiration rate of small crumbs was similar to that of large ones (Table 1). Anaerobic decomposition may have taken place in the centres of the crumbs forming simple organic compounds which then diffused to the outer surfaces where oxidation took place. Such processes, by providing readily oxidizable substrate, would have increased the respiration rate in the aerobic portion of the crumbs to values greater than could be accounted for in Eq. (4). The possibility was eliminated because the respiration rates of soil crumbs that had been pre-percolated under nitrogen were similar to the respiration rates of soil crumbs that had received no pre-percolation (Fig. 2). Since many of the products of anaerobic decomposition are readily decomposed under aerobic conditions (Table 5);

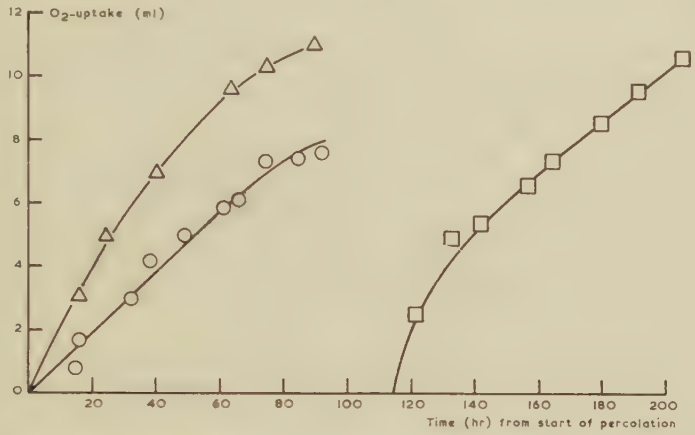

Fig. 2. Oxygen uptake consequent on percolating water through 10 g of soil crumbs; –△– under an oxygen partial pressure of 15.1 cm of mercury; –○– under an oxygen partial pressure of 1.8 cm of mercury; –□– under nitrogen for 116 h and then under an oxygen partial pressure of 15.1 cm of mercury. Crumbs were of soil A and when water-saturated they had a mean square of the radius (R^2) of 2.4 × 10^{-2} cm^2 and contained 59% water (v/v).

TABLE 2

Effect of oxygen partial pressure on the respiration of soil crumbs in Warburg flasks

Crumbs of soil A were pre-percolated for 6 months, with water in forced air percolators [8]. After drying to about 13 per cent water content (v/v) they were transferred in 1.5-g lots to Warburg flasks, re-moistened with 0.9 ml of water, the oxygen partial pressures in the flasks altered and oxygen uptake measurements made over a period of 12 h. Respiration rate was 0.035 µl/min/ml of soil crumbs under air. Oxygen concentration in centre of crumbs was found from Eq. (2) with $R^2 = 2.4 \times 10^{-2}$ cm^2 and $D = 1 \times 10^{-5}$ $cm^2 sec^{-1}$. If respiration of soil crumbs under air is taken as 100 then S.E. is ± 1.7 (4)

Oxygen partial pressure in cm of mercury (mean over period of experiment)	Mean oxygen concentration at outside surfaces of crumbs µ*M*	Mean oxygen concentration at centre of crumbs µ*M*	Percentage inhibition of respiration
0.43	7.0	0	17
0.76	12.4	1.2	0
0.87	14.3	3.1	−3

Fig. 2 also showed that the anaerobic decomposition of soil organic matter was very slow.

Since Eq. (4) now appeared valid, more accurate results were obtained by applying it to slowly respiring crumbs. To reduce the

rate of respiration, soils were pre-percolated with water under an atmosphere of air for six months. With such soils there was no measurable inhibition of respiration even at oxygen concentrations of 3×10^{-6} M (Table 2).

That the above findings applied to a wide range of soils and thus organic materials, was indicated by an experiment in which diffusion of oxygen through water was minimized by partially drying the soil. Under these conditions there was less than 20 per cent inhibition of respiration when the oxygen partial pressure was 0.8 cm of mercury (Table 3).

The slow rate of anaerobic decomposition of soil organic matter precluded its use to test for anaerobic metabolism. Some amino acids have hitherto [7] been found to decompose under anaerobic conditions and were therefore tested singly and in mixtures for rapid anaerobic decomposition. Casein digest was found to decompose most rapidly (Table 4) and in consequence might have been expected to show the greatest tendency to give anaerobic products at reduced oxygen concentrations.

The effect of oxygen concentration on the respiration of soils that had been pre-percolated with casein digest was determined by using the known relation between oxygen concentration and yeast

TABLE 3

The effect of oxygen concentration on the respiration of different soils at low moisture contents

The fraction of air-dried soil passing through a sieve having a 2-mm diam. mesh was moistened and transferred to Warburg flasks. Oxygen uptake measurements were made after replacing the atmosphere with known amounts of oxygen and nitrogen. Respiration rate indicates the oxygen uptake μl/min/ml of moist soil when the oxygen partial pressure was 16 cm of mercury. Percentage inhibition of respiration indicates the inhibition resulting from lowering the oxygen partial pressure from 16 cm to that indicated. Manometric readings were taken over a period of from 200 to 500 min. Average S.E. for percentage inhibition of respiration is ± 5 (3)

Soil	Respiration rate	Percentage inhibition of respiration	Oxygen partial pressure in cm of mercury (mean over period of experiment)	Percentage moisture content (v/v)
A	0.37	13	0.8	33
B	0.13	0	0.8	40
C	0.17	14	0.9	41
D	0.39	20	0.9	53

TABLE 4

Decomposition of amino acids under nitrogen

Crumbs of soil A (10 g) were percolated under air with either water (100 ml) or with a solution (100 ml) containing 1 mg/ml of the indicated amino acid(s) at 25°C. After 5 days, when all the α-amino-N had disappeared, both sets of soils were transferred in 2-g amounts (dry weight basis) to test tubes and 5 ml of a solution containing 15 mg of the same amino acid(s) as that used for percolation were added. The tube was sealed with a vaccine closure [6] and the air replaced by nitrogen. The figures indicate the percentage of α-amino-N decomposed after 72 h of incubation at 25°C. Mixture consists of equal proportions of alanine, aspartic acid, glutamic acid and serine. Average S.E. is ± 6 (2)

Amino acid(s)	Percentage decomposition	
	Soil pre-percolated with water	Soil pre-percolated with solution of amino acid(s)
Alanine	25	21
Aspartic acid	0	5
Glutamic acid	51	69
Serine	38	59
Mixture	55	67
Casein digest	77	80

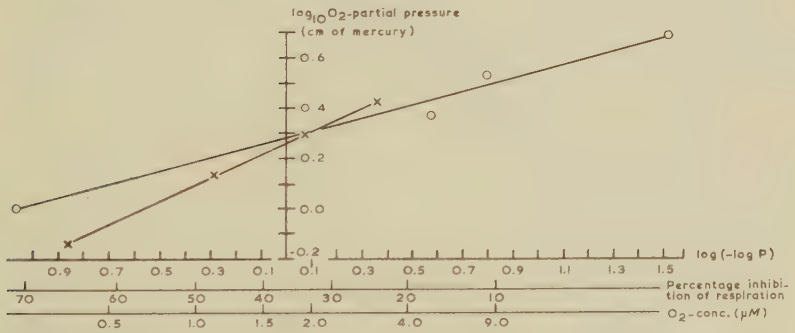

Fig. 3. Comparison of the respiration rates of casein digest-stimulated soil—water mixtures —×—, with those of yeast—sterilised soil—water mixtures —○—. Conditions were described in Experimental Section. Percentage inhibition is the inhibition of respiration expressed as a percentage of the respiration when the oxygen partial pressure was 16.0 cm of mercury. Log (—log p) was estimated from tables [12] where p is equal $10^{-2}\times$ percentage inhibition of respiration. Line of best fit with casein digest is $y = 0.46 + 0.27$ and with yeast it is $y = 0.26x + 0.28$. The oxygen concentration was obtained from the known relation [10] between oxygen concentration and yeast respiration and from an estimated K_M for yeast of 10^{-6} M.

respiration. By using the transformation $y = \log(-\log p)$, tabulated by Mather [12], a linear function was obtained between the oxygen partial pressure and the respiration rate, in Warburg flasks, of both casein digest-stimulated soil—water mixtures and yeast—sterilized soil—water mixtures. From Fig. 3, the oxygen partial pressure at which the respiration of casein digest stimulated soils was 30 per cent inhibited had a $\log_{10}$ of 0.35 cm of mercury. At this partial pressure the respiration of yeast was approximately 27 per cent inhibited. Such inhibition is known to occur when the oxygen concentration in the water surrounding the organisms is 2.7 multiplied by their Michaelis constant (K_M) [10]. The K_M (molar of oxygen) of yeast at 19°C is 6.4×10^{-7}, of *B. megatherium* at 20°C it is 7.1×10^{-7}, and of *B. megatherium* at 32°C it is 2.8×10^{-6}. It may be argued therefore that the K_M of yeast at 25°C is about 1×10^{-6} *M*. Thus an oxygen concentration of 2.7×10^{-6} *M* caused a 30 per cent inhibition of the respiration consequent on the addition of casein digest to soil.

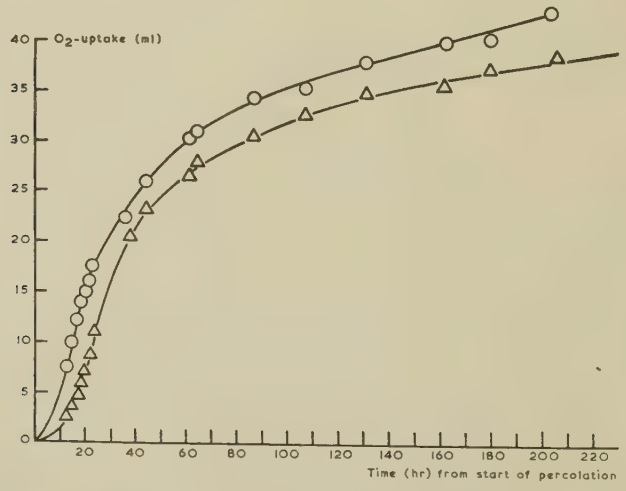

Fig. 4. Oxygen uptakes consequent on percolation of casein digest containing 3.2 mg of α-amino-N through 10 g of soil crumbs when the oxygen partial pressure was 15.3 cm of mercury –○–; and when the oxygen partial pressure was 2.0 cm of mercury –△–. Respiration rates of soils to which no casein digest had been added were as shown in Fig. 2. Crumbs were of soil A and when water-saturated they had a mean square of the radius (R^2) of 2.4×10^{-2} cm^2 and contained 59% water (v/v).

When casein digest solution was percolated through soil crumbs held under oxygen partial pressures of 15.3 and of 2.0 cm of mercury, the oxygen uptakes were similar (Fig. 4). After 90 h percolation no α-amino N could be detected in any of the percolators; under nitrogen 81 μe of volatile fatty acid were produced per mg of α-amino-N; but under neither of the oxygen partial pressures could more than 2 μe of volatile fatty acid be detected. At this stage the entire percolates were transferred to fresh samples of soil A and the oxygen uptakes measured. Percolates which had been under either of the oxygen partial pressures gave no oxygen uptake additional to that of the moistened soil, whereas percolates which had been under nitrogen gave oxygen uptakes identical with

TABLE 5

Effect of oxygen partial pressure on the decomposition of simple organic compounds

The indicated materials were percolated through soil crumbs held under different oxygen partial pressures. Theoretical oxygen uptakes were calculated assuming complete conversion of substrate into CO_2, H_2O and NH_3. K is defined as $1/t \log_{10} C/C - c = K$, where C = oxygen uptake obtained after 120 h, c = oxygen uptake after time t; the oxygen uptakes resulting from the endogenous respiration were assumed equal to the oxygen uptakes obtained in untreated soils at the given oxygen partial pressures. Soil A was used and the temp. was 25°C except in experiments with alanine where soil E was used and a temp. of 21°C. Products of casein digest refer to what appeared in the percolate after percolating casein digest through 10 g of soil A under N_2 for a period of 4 days by which time all the α-amino-N had disappeared

Substrate	Theoretical O_2-uptake at N.T.P.	O_2-partial pressure cm of mercury	Percentage of theoretical O_2-uptake 90 h after start of percolation	$10^{+6} \times K$ sec^{-1}
Alanine	15	15.3	66	3
Alanine	15	1.6	60	4
Acetic acid . . .	44	1.7	78	5
Butyric acid . .	45	1.7	72	5
Formic acid . . .	26	1.7	76	4
Propionic acid .	48	1.7	54	3
Valeric acid . . .	52	1.7	58	3
Casein digest . .	36	15.3	56	6
Casein digest . .	36	2.0	55	4
Products of casein digest	36	15.3	53	7
Products of casein digest	36	2.0	51	4
Average S.E. . .	—	—	± 5 (2)	± 0.5 (2)

those obtained with undecomposed casein digest (Table 5). In experiments where the atmosphere had an oxygen partial pressure of about 2.0 cm of mercury, more than 80 per cent of the soil provided conditions suitable for anaerobic decomposition (Eq. 4). It must have occurred since casein digest was found to lose 25 per cent of its α-amino-N during the first 24 h of anaerobic percolation. A possible explanation for the inability to detect any products of anaerobic decomposition is that they diffused into regions where there was sufficient oxygen to maintain aerobic metabolism. This was supported by other experiments which demonstrated that products of anaerobic metabolism, including volatile fatty acids, rapidly decomposed under a wide range of oxygen partial pressures. For example, Table 5 shows that the products of casein digest anaerobic decomposition and volatile fatty acids gave oxygen uptakes after 90 h of percolation, and K-values [9], which were independent of oxygen partial pressure, between 15.3 and 1.7 cm of mercury. Moreover the K-values obtained were $3.7 \times 10^{-6}\text{sec}^{-1}$ and thus were of the same order as those previously obtained for the aerobic decomposition of similar compounds [5 9]. The possibility that aerobic metabolism would have been inhibited by the reduced volume of soil in which it could have taken place was eliminated by the findings that similar oxygen uptakes were obtained by the percolation, under air, of casein digest solution (3 mg α-amino-N/50 ml) both through 10 g and through 2.5 g of soil crumbs.

DISCUSSION

It appears that the major assumptions (Eq. 1–5) used in the work on the respiration of water saturated soil crumbs were valid (Figs. 1 and 2 and Table 1). The oxygen concentration, C_0, at which respiration was assumed to cease, approximates to the concentration at which respiration is 50 per cent inhibited. The value obtained for C_0 was less than $3 \times 10^{-6}\ M$ (Fig. 1 and Table 2) and appeared to be similar in soils of widely different type (Table 3). Since the decomposition of complex organic materials under both aerobic and anaerobic conditions must proceed via the formation of simple organic compounds which are then further metabolized, the results of the work with these compounds is significant. Respiration resulting from the addition of casein digest to soil was

30 per cent inhibited at an oxygen concentration of 2.7×10^{-6} M (Fig. 3). Moreover, the decomposition under reduced oxygen partial pressures of all the simple compounds tested was similar to that of casein digest (Table 5), so that by analogy the effect of oxygen concentration on decomposition would be similar. These findings, coupled with the fact that none of the simple compounds were detected after 72 h percolation at an oxygen partial pressure of 1–2 cm of mercury (Table 5), indicate that none could have accumulated until the oxygen concentration was less then about 3×10^{-6} M.

Results of work with other systems appear at least consistent with these findings. Longmuir [10], using ingenious polarographic techniques, studied the effect of oxygen concentration on the respiration of some pure cultures of micro-organisms. He found that in all cases the relation between oxygen concentration and respiration rate fitted the Michaelis-Menten equation and that the values for K_M (molar oxygen) obtained were between 1×10^{-6} and 1×10^{-8}. The oxygen concentration of water-saturated soil was shown by Scott and Evans [17] to fall rapidly to zero. In their system the rate of loss of oxygen was constant until an oxygen concentration of about 5×10^{-5} M, and it may well have been lower had it been possible to use a more sensitive technique. Most of the other work using soils has been confined to studying the effect of oxygen partial pressure [1 13] on the decomposition of various straws. Some inhibition of respiration was found even at an oxygen partial pressure as high as 4.0 cm of mercury. Inhibition, however, may have been the result of localised anaerobic pockets. The explanation depends on there having been a slower rate of anaerobic than aerobic decomposition which is verified by Table 4 and Fig. 2 and also by work described elsewhere [7 13].

Dean and Hinshelwood [3] have shown that adaptation in pure cultures of organisms can be explained by change in enzymic activities taking place in such a way as to keep the total growth rate at the highest possible value. In soil, changes in enzymic activities are brought about by conditions very different from those obtaining in pure cultures of organisms; for example changes may take place by selection as well as by adaptation. Yet the theorem of Dean and Hinshelwood fits all the microbial changes in soil described in this and in previous papers [5 7 8]. Conversion

of organic substrates into cell material requires energy which may be obtained by transfer of hydrogen from some of the substrate, either to another molecule of substrate (*1*) or to oxygen (*2*). Process (*1*) gives volatile fatty acids amongst the products. Since (*2*) gives more energy per unit of substrate than (*1*) there will also be more cell synthesis. Free-energy changes in chemical reactions are little affected by reduction in oxygen concentration so that even at low oxygen concentrations (*2*) will take place in preference to (*1*). The total metabolizing activity will increase with increase of cell synthesis and thus will be greater in the presence of oxygen than in its absence. There will, therefore, be a much slower overall decomposition of organic materials in the absence than in the presence of oxygen (Cf Table 4, Table 5 and Fig. 2). Likewise an explanation can be given for earlier findings that volatile fatty acid formation in soil held under anaerobic conditions was suppressed by nitrates [7 11]; nitrate would be used preferentially to the organic substrate as a hydrogen acceptor.

There are then, good reasons for the following hypothesis: the decomposition of organic materials in the presence of oxygen, is accompanied by the formation of large quantities of cell material and is not inhibited through shortage of oxygen unless the oxygen concentration is less than about 3×10^{-6} *M*. At lower oxygen concentrations decomposition is slow, but where it does take place large quantities of volatile fatty acid and other simple organic compounds accumulate.

The fact that respiration was not inhibited by lack of oxygen until very low oxygen concentrations has many implications besides those already discussed. For instance it means that the distribution of oxygen in any soil system can be determined by using the respiration of soil as an indicator of oxygen. It has been shown by this means that water-saturated crumbs of soil A greater than 3 mm in radius had no oxygen at their centres. Since crumbs of this size and in this condition are present in most soils, it means that localised pockets of anaerobic conditions are widespread and thus provides amongst other things, an explanation for the universal occurrence of strict anaerobes.

SUMMARY

1. Techniques are described for relating the oxygen concentrations in the soil water on the surfaces of micro-organisms to their metabolizing activities.

2. Studies were made on the decomposition of organic materials in water-saturated crumbs (mean radius 1.55×10^{-1} cm) of a loam soil.

3. Respiration of water-saturated crumbs was not inhibited unless the oxygen concentration was less than about 10^{-6} *M*. Evidence was obtained that above a similar low oxygen concentration there was no inhibition of respiration in soils of widely different type.

4. Anaerobic decomposition of the soil organic matter was very slow. Anaerobic decomposition of casein digest was more rapid than that of any other material tested; the products were water soluble and included 83 μ-equivalents of volatile fatty acid per mg of α-amino-N decomposed.

5. Casein digest percolation of soil crumbs under air resulted in the formation of micro-organisms that respired at 70 per cent of their maximum rate when the oxygen concentration was about 2.7×10^{-6} *M*.

6. No products of anaerobic casein digest decomposition could be detected on percolating casein digest through soil crumbs when 80 per cent of the soil contained no oxygen and the maximum concentration in any part of the soil was about 3×10^{-5} *M*.

7. The kinetics of oxygen uptake consequent on the decomposition of casein digest and of other simple organic compounds in soil crumbs were similar and were only slightly affected by reduction of oxygen partial pressure in the atmosphere from 15 to 1.7 cm of mercury.

8. It is concluded that 'change-over' from aerobic to anaerobic metabolism of organic materials takes place in widely different soils at an oxygen concentration less than about 3×10^{-6} *M*.

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IODINE AS A MICRONUTRIENT FOR PLANTS

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INTRODUCTION

Up till now it has not been possible from a study of the literature to draw definite conclusions concerning the effect of iodine on plant growth. Some authors (Aso and Suzuki [2], Buzanov and Popova [4], Powers [19], Stoklasa [27 28], Suzuki [31], and Suzuki and Aso [32]) have found that iodine has a distinctly positive influence whereas others (Fellenberg [9], Lewis and Powers [14], Newton and Toth [18], and Scharrer and Schropp [22]) have not noted any beneficial effect. Many of these investigations relate to experiments in which iodine was applied to the soil. According to observations by Lehr *et al.*[13] and Verona and Ferretti [33] iodine can stimulate nitrification in the soil and may thus have an indirect effect on plant growth. It is, therefore, not *a priori* certain that increases in yield which have been produced by iodine fertilization of the soil should be attributed to a physiological effect of iodine in the plant. Of the investigators who excluded the soil factor in their experiments, some (Ehling [7], Meyer [16], Scharrer and Schropp [23], and Stoklasa [28 29]) concluded that iodine can stimulate the growth of plants whereas others (Hageman *et al.*[11], Muckenhirn [17], Newton and Toth [18], and Wynd [36]) reported no significant effect or even a negative one.

Various reasons can be put forward for these contradictory results. As has been shown by the Chilean Iodine Educational Bureau (London) [5] in an extensive review of the literature on the role of iodine in plant growth, a positive effect can only be expected at

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low doses of iodine, and most of the negative results recorded in the literature are due to an overdosage of iodine. Lehr *et al.*[13] carried out physiological experiments with tomato plants during 1952 to 1955 and found that the development of this crop was clearly stimulated by iodide if the concentration used was not too high. The nature of the crop is important in so far as various investigators have shown that some crops are more favourably influenced than others. The form in which iodine is applied is also of importance since a study of the literature indicates that plants can withstand higher concentrations of iodate than of iodide (see also 'Discussion').

These considerations prompted us to study in our investigation both the effect of the concentration and of the form of iodine on the growth of a number of crops of agricultural importance. In addition we determined the uptake of iodine by several plant species in order to ascertain whether any correlation exists between the reaction of plants to different forms of iodine and their iodine content.

EXPERIMENTAL TECHNIQUE

The pot experiments described were carried out in 1956 and 1957 and the division of crops over these two years is shown in Table 2. Buckwheat, tomatoes, mustard, turnips, and spinach were grown in 6.9-litre pots, and flax, white clover, barley and perennial ryegrass in 2.5-litre glass cylinders. Fodderbeet was cultivated in 17-litre pots and wheat in plastic pots of 1.3 litres capacity. The type of pots used are those described by Schuffelen *et al.*[24]

The pots were filled with pure sand mixed with nutrient salts, and a germination layer of pure sand only was left on the surface, thus:

Type of pot	Total quantity of sand per pot, kg	Quantity of sand in germination layer, kg
Plastic pots	1.8	0.3
Glass cylinders	3.0	0.3
Enamelled pots (6.9 litre)	7.5	0.5
Enamelled pots (17 litre)	18.0	1.0

The pots were kept at 60 per cent of their water-holding capacity and watered two or three times a day with demineralized water. The number of replications for most trials was three, but for mustard (1957) it was four and for fodderbeet five. All crops, except mustard (1957), were harvested at the vegetative stage.

TABLE 2

Dry weight of crops expressed as per cent of control; dry weight of control in g per pot; iodine content in mg per kg dry weight. All data refer to aerial parts of plants unless otherwise stated

Crop		Dry weight as per cent of control								Dry weight	Iodine content (mg I/kg)		
		I as iodide, ppm				I as iodate, ppm						Highest iodine dose	
1956 Experiments		0.02	0.04	0.08	1.00	0.02	0.04	0.08	1.00	Control	Control	KI	KIO_3
Buckwheat *		102	96	101	74	92	94	92	87	13.2	1.3	180	74
Tomatoes *		109	114	112	86	113	123	114	118	16.0	1.26	153	72
White clover *	1st cut	55	115	97	100	–	118	124	91	3.3	1.01	233	112
	2nd cut	59	116	88	97	–	109	84	78	3.2	0.73	39	73
	3rd cut	81	119	99	119	–	108	111	100	7.2	0.40	4.9	14.3
Flax **		72	105	114	80	–	96	106	84	9.5	0.81	137	87
P. ryegrass *	1st cut	97	125	117	111	111	125	125	125	3.6	1.36	61	26
	2nd cut	82	108	102	98	96	102	102	106	4.9	0.48	9.7	7.8
	3rd cut	98	114	105	108	116	119	124	118	6.2	0.34	0.57	2.43
	4th cut	91	100	98	89	89	92	96	94	5.3	0.63	0.60	1.08
Mustard *		102	88	109	85	105	105	113	84	12.9	0.7	174	92
Barley		103	103	90	105	107	101	101	101	14.9	0.42	28	12
1957 Experiments		I as iodide, ppm				I as iodate, ppm							
		0.01	0.04	0.16	0.64	0.01	0.04	0.16	0.64				
Wheat		95	105	90	96	90	108	107	107	13.2	0.61	3.9	4.0
Oats		94	87	86	55	96	90	95	87	10.2	0.30	20	4.7
Spinach §		105	102	102	95	105	102	110	98	6.3	10 §	360	270
Mustard §§		94	97	93	97	101	102	95	95	14.9	n.d.	n.d.	n.d.
P. ryegrass †	1st cut	107	107	102	110	114	110	114	112	4.2	0.30	7.9	4.5 †
	2nd cut	94	92	94	97	94	92	89	88	6.5	0.28	0.85	4.6
	3rd cut	100	102	107	100	95	102	100	98	5.6	0.35	2.9	4.1
Turnip	leaves	94	105	109	104	102	105	104	105	11.6	0.91	77	48
	roots	91	95	90	78	87	93	84	97	15.1	0.31	4.9	16.4
Fodderbeet ††	leaf blades	101	93	123	140	107	117	124	123	8.8	2.4	620	390
	stalks	102	104	125	142	96	102	93	85	5.5	2.4	530	540
	roots	81	103	94	141	85	103	96	73	21.4	0.62	32	76

* Only 4/6 of iodine dose applied.
** Only 5/6 of iodine dose applied.
§ Some contamination of control samples occurred, probably after harvesting.
§§ Harvested at reproductive stage; dry weight of stems without leaves.
† Iodate doses were only 72 per cent of those indicated. After 2nd cut an extra dose, equal to that indicated for both KI and KIO_3, applied.
†† 48 days after emergence of plants second iodine dose, equal to first, applied.

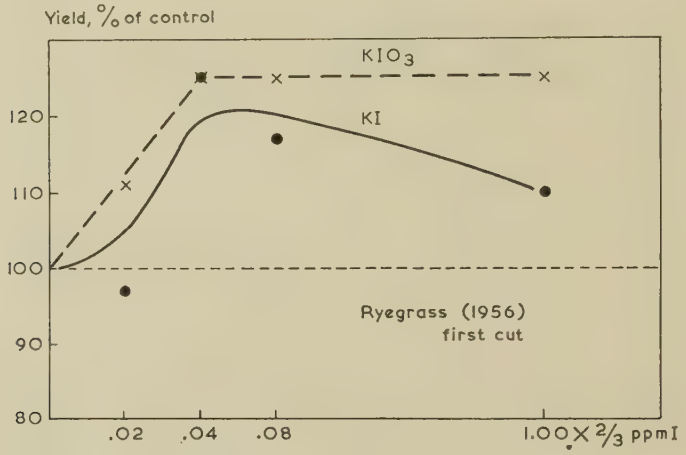

Fig. 2. Illustration of the effect of iodide and iodate on the dry-matter yield of perennial ryegrass.

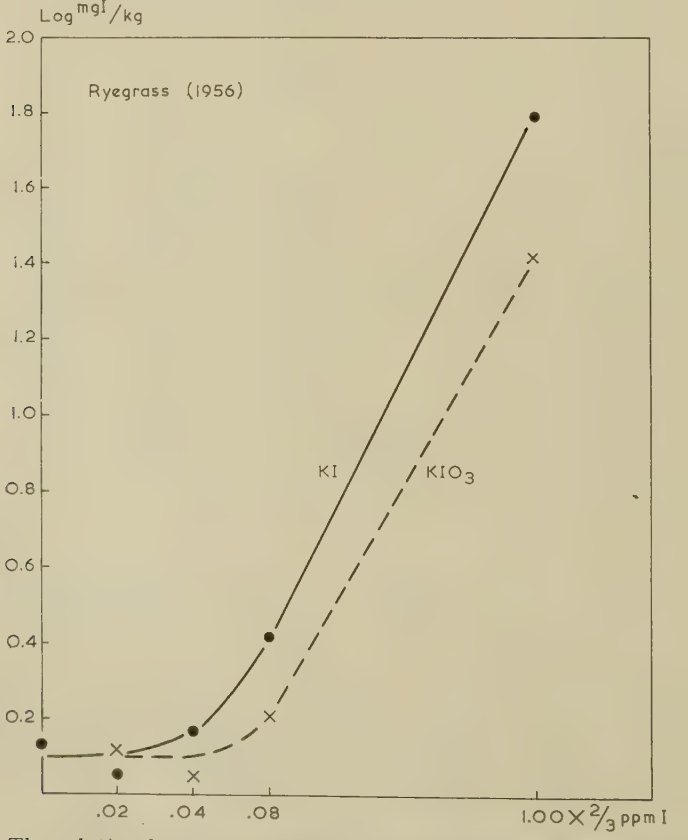

Fig. 3. The relation between the logarithm of the iodine content (expressed in mg/kg dry weight) of the first cut of perennial ryegrass and the added dose of KI or KIO_3.

Fertilization

The doses of nutrients applied per kg sand are given in Table 1:

TABLE 1

Doses of nutrients applied, mg per kg sand					
Nutrient salt	1956	1957	Nutrient salt	1956	1957
$CaHPO_4.2H_2O$	333*	33*	$FeCl_3.6H_2O$	33	–
$Ca(H_2PO_4)_2$	–	145	Fe as versenate	–	6.7
KCl	83	25**	$CuSO_4.5H_2O$	8.3	6.7
K_2SO_4	–	240**	$ZnSO_4.7H_2O$	1.7	3.3
NH_4NO_3	47	47***	$MnSO_4.4H_2O$	17.7	13.3
$NaNO_3$	100	100***	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.7	0.7
$MgSO_4.7H_2O$	100	133	$Na_2B_4O_7$	3.3	6.7***

* The sand used in the experiments in 1956 was somewhat contaminated with $CaCO_3$ which caused the pH to be too high and led to manganese deficiency. To prevent a recurrence in 1957 we partly replaced the almost neutral $CaHPO_4.2H_2O$ by the more acid $Ca(H_2PO_4)_2$.

** The amount of chloride was substantially decreased in 1957 as, from other pot trials, we had some indications that high doses of chlorides can depress the development of crops. Later experiments, however, did not confirm this experience.

*** Fodderbeet received 142 mg NH_4NO_3, 300 mg $NaNO_3$ and 40 mg $Na_2B_4O_7$ per kg sand.

The amounts of KCl (1956), $NaNO_3$ (1956 and 1957) and NH_4NO_3 (1956 and 1957) given in Table 1 do not include the amounts given as a topdressing (mostly two or three times the corresponding basal dressing).

Iodine doses

Iodine was applied in two forms, namely, potassium iodide and potassium iodate. The doses planned for the 1956 experiments were 0.02, 0.04, 0.08, and 1.00 ppm I, to be applied in six portions, but most of the plants received only four or five portions. In 1957 the iodine doses amounted to 0.01, 0.04, 0.16, and 0.64 ppm. These were applied at the time of filling the pots, but a second application was later given to fodderbeet and perennial ryegrass.

The iodine content of the sand in the control pots was kept as low as possible; in 1956 it was not more than 0.01 ppm and in 1957 was even less than 0.003 ppm. Only reagent-grade salts were applied and all the water used was demineralized.

Method for iodine determination

The method followed was a modification of one described in a publication of the Chilean Iodine Educational Bureau (London) [6], in which plant samples are digested with potassium permanganate and sulphuric acid. The iodate formed in the digestion mixture is reduced with oxalic acid and the iodine liberated is distilled into an alkaline solution and is then determined colori-

metrically by means of the catalytic effect of iodide on the reduction of ceric sulphate by arsenious acid.

RESULTS

Effect of iodine on development and yield

During the early stages of development the plants generally responded better to KIO_3 than to KI. The highest dose of KI and, to a lesser extent, of KIO_3, however, depressed the initial growth in several species.

Application of iodine increased both the longitudinal and the radial growth of tomato plants, as well as the radial growth of mustard. Furthermore the leaf surface of treated mustard plants was greater than that of controls. In the 1957 experiments with oats and wheat iodine doses of about 0.1 ppm advanced earing by a few days. The budding of mustard, on the other hand, was somewhat retarded by iodine.

As shown in Table 2 iodine, both as iodide and iodate, can have a positive effect on vegetative growth (see also Figures 1 and 2). In several crops the dry-matter content increased by 5 to 25 per cent and in fodderbeet by as much as 40 per cent. Only the growth of oats was retarded by all the iodine doses applied.

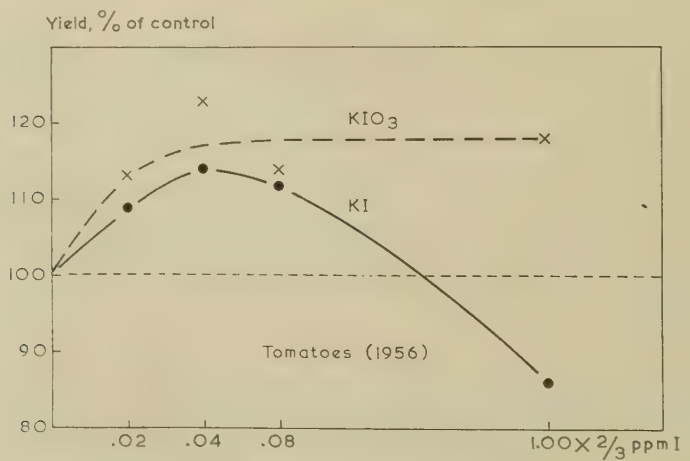

Fig. 1. Illustration of the effect of iodide and iodate on the dry-matter yield of tomatoes.

Iodine content of plants

The iodine content of most of the plants which received the highest iodine dose was higher for the KI than for the KIO_3 treatment (see Table 2). The relation between the iodine content of perennial ryegrass (1956, first cut) and the various doses of iodine applied is shown in Figure 3.

DISCUSSION

Effect of iodine on planth growth

The experiments described above clearly show that iodine can exercise a favourable effect on the vegetative development of several species of plants in sand cultures. The growth of spinach, white clover, fodderbeet, tomatoes, perennial ryegrass and of turnips (aerial parts only) was significantly stimulated, while that of barley, flax, wheat and mustard was, in general, much better following the application of minute quantities of iodine as iodide or iodate. No apparent stimulation was shown by buckwheat and the growth of oats and to a lesser extent also of turnip roots was even retarded at all the doses applied. It may be stated here, however, that a certain amount of stimulation of oats by iodine has been observed by us in water-culture experiments. It is interesting to point out that the growth of white clover and flax was markedly depressed by the lowest dose of iodide, a fact which we are unable to explain.

We gained the impression that the plants of our 1956 experiments generally responded somewhat better to iodine than those of our 1957 experiments. This may have been partly due to the fact that in 1956 the iodine was applied in small portions whereas in 1957 the whole dose was applied at once. Another factor which may have been involved was the higher dose of potassium chloride used in 1956 which may have had a favourable influence on the iodine effect as has been shown by Lewis and Powers [15] in maize.

As stated in the 'Introduction', it was already known from a study of the literature that not all plant species react in the same manner to iodine and that the effect appears to be greatly dependent on the nature of the crop. Ehling [7] has observed that the growth of mustard and spinach is favourably influenced by iodide, whereas, at the same dosage, the development of oats is hindered. Scharrer

and Schropp [20 21] have also shown that oats are much more sensitive to iodine than other cereals. Wheat, according to the same authors, occupies an intermediate position between barley and oats, a fact which we were able to confirm in our own investigations. The stimulation of the growth of wheat noted by us has also been observed by Åberg [1] and Ekdahl [8] in experiments with seedlings.

Stoklasa [29] is of the opinion that halophytes respond better to iodine than do other crops. This is in agreement with our observation that fodderbeet shows a very favourable reaction even at high doses of iodide.

Our observation of the increased growth of tomatoes is in accord with the earlier findings of Lehr *et al.*[13] In this connection mention should also be made of the investigations of Hannay [12], Street [30] and White [34] on the culture of excised tomato roots from which it appears that iodine as iodide markedly stimulates the development of the roots.

A comparison of our data with those reported in the literature (see Table 3) shows clearly that various crops differ in their sensitivity to iodine. Some plants can easily withstand a dose of 0.1 to 1.0 ppm I whereas with others only doses of 0.01 to 0.10 ppm I have a favourable effect. In some species even these latter doses are toxic.

From its very nature also the optimum dose of iodine varies for different crops. For most of the crops investigated, as far as they showed a positive effect, the optimum dose varied from 0.025 to 0.2 ppm I. In a few crops, however, it was as high as 0.6 ppm I.

It is difficult to say whether iodine, in addition to having a general and stimulating effect on the development of plants, also exercises some specific function. Lehr *et al.*[13] found that it stimulated tangential growth at the expense of radial growth but we were unable to confirm this since we observed an increase in both. The advancement of earing in wheat and oats possibly indicates a specific effect. This should not be considered as emergency flowering, however, since the effect was more pronounced with iodate, which has a more favourable effect on growth, than with iodide.

We observed a stimulating effect of iodine on leaf growth in both a sand-culture experiment with mustard and a water-culture experiment (unpublished) conducted under controlled conditions with white clover. In the latter experiment the salts used for the nutrient solution were prepared by repeated recrystallization, which

TABLE 3

Comparison of results obtained in the present investigation with data reported in the literature

Crop	Effect on yield				Better response	Slower uptake
	KI series		KIO_3 series			
	0.01–0.1 ppm I	0.1–1.0 ppm I	0.01–0.1 ppm I	0.1–1.0 ppm I		
Fodderbeet leaf blades	0	+	+	+	KI	KIO_3
Fodderbeet roots	—	+	—	—	KI	KI
Perennial ryegrass	+	+	+	+	KIO_3	KIO_3
Barley	0	+	+	0	~	KIO_3
Wheat	0	—	0	+	KIO_3	~
Wheat (Ref.[1])					KIO_3	
Tomatoes	+	—	+	+	KIO_3	KIO_3
Spinach	+	0	+	+	KIO_3	KIO_3
Spinach (Ref.[7])	+	—	+	+	KIO_3	
White clover *	+	0	+	—	KIO_3	KIO_3
Mustard	0	—	+	—	KIO_3	KIO_3
Mustard (Ref.[7])	+	—	+	—	KIO_3	
Flax *	+	—	0	—	KI	KIO_3
Turnip leaves	0	+	+	+	~	KIO_3
Turnip roots	—	—	—	—	KIO_3	KI
Buckwheat	0	—	—	—	KI	KIO_3
Oats	—	—	—	—	KIO_3	KIO_3
Oats (Ref.[7])	—	—	+	+	KIO_3	

* The effect of the lowest dose of iodine is not included.

The data for the items italicized are taken from the literature.

+ = positive effect; — = negative effect; 0 = no effect

~ = no pronounced difference between KI and KIO_3

decreased their iodine content by 60 per cent, and the air was, as far as possible, freed from iodine.

It is important to note that analyses of the control plants showed relatively high values for iodine content. Most probably this must be partly ascribed to the fact that they were cultivated in the same space as the treated plants and were thus exposed to an atmosphere fairly rich in iodine. Since several investigators (Ehling [7], Gaus and Griessbach [10], Selders [25], Selders and Hungate [26], and Wrangell [35] have demonstrated that plants are able to absorb iodine from the air, it is to be expected, therefore, that in an iodine-free atmosphere the iodine content of the control plants will be lower and the difference in growth between the control and the iodine-treated plants will be greater.

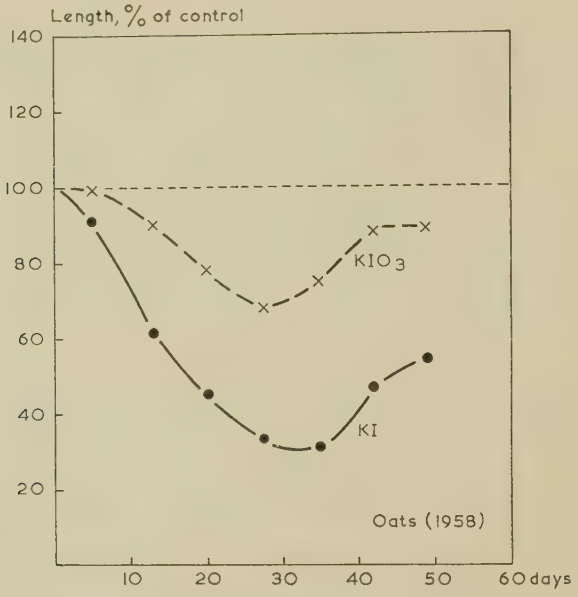

Fig. 4. Effect of iodine (1 ppm I, either as KI or KIO_3) on the trend of the longitudinal growth of oats expressed as percentage of the control.

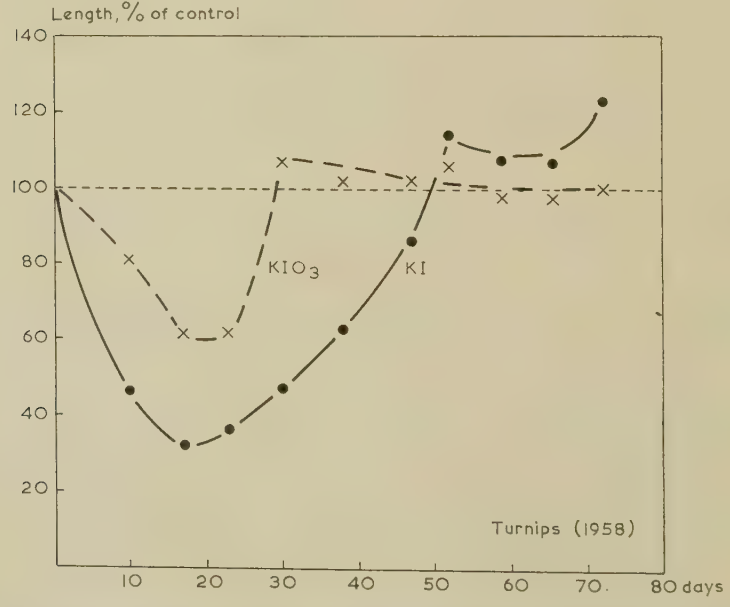

Fig. 5. Effect of iodine (10 ppm I, either as KI or KIO_3) on the trend of the longitudinal growth of turnips expressed as percentage of the control.

Relation between rate of uptake of iodide and iodate and their effect on plant growth

The difference observed between iodide and iodate in their effect on plant growth may be partly explained by the different rates of uptake of these salts. A high rate of uptake will especially affect the initial development of the plant since a high concentration of iodine instead of exercising a stimulative effect can lead to a temporary retardation of growth. This retardation will thus be greater with iodide than with iodate. An illustration of the difference in effect between iodide and iodate during the initial growth of oats and turnips is given in Figures 4 and 5. It is apparent that the retardation of growth in these crops was much more pronounced in plants treated with KI than in those treated with KIO_3. The results (unpublished) of an experiment carried out in 1958 demonstrated that the retardation of growth in turnip plantlets ran parallel to their iodine content (see Figures 6 and 7). After some time, however, the plants in this experiment recovered, and concurrently there was a sharp decrease in their iodine content.

Usually the difference in initial development makes itself felt at later stages of growth. This, however, is not a general rule since we have seen that in respect of dry matter yield some crops respond better to iodide than to iodate.

The foregoing explanation does not exclude the possibility of a specific effect of iodide and iodate within the plant. In point of fact the redox potentials of these ions differ greatly and thus, in this respect also, iodide and iodate will exercise a different effect on the plant.

In addition, KI and KIO_3 may have a different effect on the cell membranes. According to Ekdahl [8] iodide can weaken the cell membranes of the root hairs of wheat to such an extent that the hairs burst at the tip and thus have their growth retarded. Iodate, on the other hand, does not exhibit this phenomenon.

Effect of form of iodine

It has been shown by earlier workers, namely, Scharrer and Schropp [20 21] for cereals, Åberg [1] and Ekdahl [8] for wheat, and Ehling [7] for oats, spinach and mustard, that certain crops respond more favourably to iodate than to iodide. In our investigations we found a better response to iodate in wheat, oats, tomatoes, mustard,

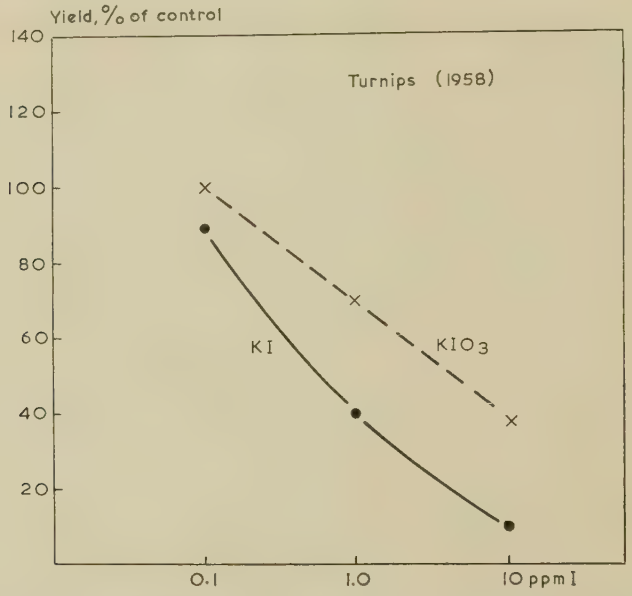

Fig. 6. The relation between the dry matter yield of the leaves of turnip plantlets 7 days after emergence (in per cent of the control) and the added dose of KI or KIO_3

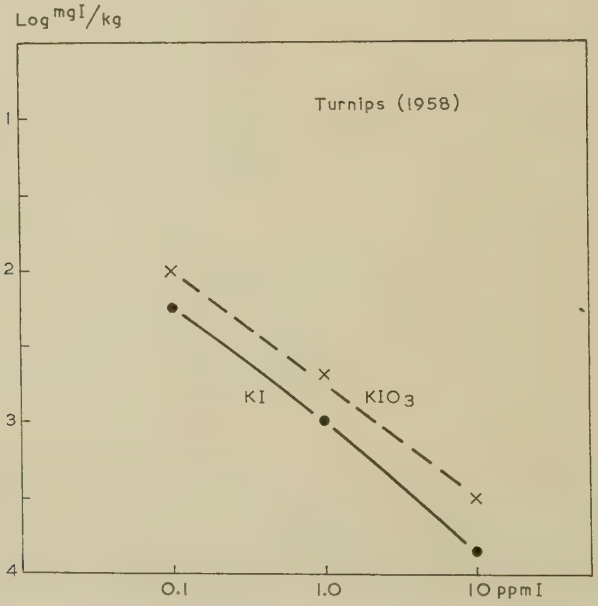

Fig. 7. The relation between the logarithm of the iodine content (expressed in mg/kg dry weight) of the leaves of turnip plantlets 7 days after emergence and the added dose of KI or KIO_3

spinach, white clover, perennial ryegrass, buckwheat, flax, and turnips, but only at the early stages of development in the last three crops. The difference in effect between iodide and iodate was especially pronounced during the initial growth of turnips and oats as can be seen from the results of two experiments carried out in 1958 (see Figures 4 and 5).

These findings thus suggest very strikingly that iodate affects plant growth more favourably than iodide. The strongest evidence is obtained during the early stages of development but the effect is very often maintained until the final stages of vegetative growth. Only barley and fodderbeet did not show a superior response to iodate.

Iodine uptake

The fact that the iodine content of plants treated with KI is generally higher than that of those treated with an equivalent dose of KIO_3 indicates that there is a quicker rate of uptake of iodine from iodide than from iodate. Confirmation of this was obtained in a waterculture experiment with oats and rape carried out according to the Bussler [3] technique. A full account of the investigation on oats will be given in a future publication but in the meantime we reproduce in Table 4 the values obtained for the uptake of iodine.

TABLE 4

Iodine uptake of oat and rape plantlets in water-culture experiment carried out by the Bussler technique

Crop	Iodine content of nutrient solution (ppm)	Iodine uptake (μg I per reagent tube)	
		KI series	KIO_3 series
Oats, aerial parts	2.25	2.7	0.9
Oats, roots	2.25	4.4	1.9
Rape, aerial parts	0.86	13.5	2.0

Only in wheat was there no clear difference in iodine uptake but we do not consider that this indicates that wheat occupies an exceptional position.

In the roots of fodderbeet and turnips iodine uptake was greater after iodate than after iodide treatment of the plants. A possible explanation for this may be offered here. Since the withdrawal of

iodide from the sand was much greater than that of iodate the remaining concentration of KIO_3 was thus higher than that of KI. Whereas the initially-absorbed iodine was translocated chiefly to the leaves, the iodine absorbed in later stages of growth also passed into the tubers. At this stage the KIO_3 concentration in the sand was much higher than that of KI and as a result there was probably a larger uptake of iodine by the tubers from the iodate than from the iodide pots. An analogous phenomenon was seen in the second and third cuts of white clover and English ryegrass. More iodine was withdrawn from the sand by the first cut in the KI treatment and by the subsequent cuts in the KIO_3 treatment.

It is also worthy of note that the difference in iodine content between the plants of the KI and KIO_3 treatments was relatively greater in the 1956 experiments than in those of 1957. This is probably due, at least in part, to the fact that the iodine salts were added in several portions in 1956 and not in one dose as in 1957. This diminished the chances of a levelling of the iodine content, which occurs on its own after some time since the concentration of iodide in the sand decreases more rapidly than that of iodate so that the relative speed of uptake of iodine from iodide gradually decreases.

In the water-culture experiment with oats and rape plantlets (see Table 4) the difference in the rate of uptake of iodine was fairly large, namely, at least 2.3 times greater for iodide than for iodate. It is not certain, however, that both iodide and iodate ions are absorbed as such. It might also be considered that iodate must first be reduced by the roots before it can be absorbed. According to Ekdahl [8] iodide has a marked weakening effect on the cell membranes of root hairs and this might make the latter more permeable to iodide than to iodate which does not exercise such an effect.

SUMMARY

In sand-culture experiments in pots it was shown that various crops react to minute applications of iodide or iodate. The vegetative growth of spinach, white clover, fodderbeet, tomatoes, perennial ryegrass, turnips (aerial parts), barley, flax, wheat and mustard was favourably influenced by iodine. No distinctly positive effect was observed, however, with buckwheat. The development of oats and to a lesser extent of the roots of turnips was hindered at all the rates of application used.

In general, iodate had a more favourable effect on growth than iodide,

particularly in the initial stages of development. This may be due partly to the fact that plants absorb iodate more slowly than iodide, since during early life plants are highly sensitive to an overdosage of iodine.

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BORON-DEFICIENCY DIEBACK IN PINES

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INTRODUCTION

In 1954, the Provincial Forestry Officer in the Southern Highlands Province of Tanganyika reported that the terminal shoots of young, experimentally planted, pines were dying back in the cold season. There is, in this Province, much unutilised open grassland lying at about 5000 to 7000 ft. altitude which is considered particularly suitable for afforestation and trials with pines were being made for this purpose. The effect of the dieback was that the pines lost most of the growing season in forming new growth and it was feared that for this reason they would prove uneconomic. Possible causes of the dieback were drought, cold, disease, or nutrient deficiency but it was noticed that it occurred in sheltered trees in well-watered sites. No pathogens could be discovered and further there was apparently no mychorrizal deficiency. There was some connection with soil type as the condition did not occur on younger volcanic soils. In places on older soils, pines, after struggling for 7 to 8 years, suddenly overcame the disability as if their roots had then reached a layer of soil containing a needed nutrient element. There was therefore a case for investigating the nutrient status of the pines and leaf analysis was applied for this purpose.

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LEAF ANALYIS

Four bulk samples of leaves from pines of varying thriftiness were first collected, as follows: –

Species	Site	Height of trees at 4 years old	Condition of trees
1. *Pinus taeda*	Mbeya Forest Office	7 ft.	Grew well for 3 years and then died back
2. *Pinus taeda*	Mbeya Nursery (recent volcanic soil)	20 ft.	Have grown extremely well
3. *Pinus patula*	Kawetire Afforestation Area	2 ft.	Suffer badly from dieback of leading shoot
4. *Pinus patula*	Mbeya Nursery (recent volcanic soil)	20 ft.	Have grown extremely well

The results of the analysis of these samples are given in Table 1.

TABLE 1

Comparative analyses * of pine leaves from Mbeya district												
Sample	Site	N	P	K	Ca	Mg	S	Mn	Fe	Cu	B	Zn
1	Mbeya Forest Office (healthy and then dying back)	2.2	.22	1.04	.29	.13	.42	135	420	22	16	44
2	Mbeya Nursery (healthy)	1.7	.15	.75	.29	.09	.16	187	43	5	15	n.d
3	Kawetire - (with dieback)	1.9	.17	1.01	.27	.19	.10	400	245	9	**7**	**26**
4	Mbeya Nursery (healthy)	2.0	.23	1.06	.41	.18	.16	296	148	8	14	41

* See note to Table 2.

Further analyses of pine leaves were done later to confirm the above and to provide better standards for comparison. These are given in Table 2. The Moshi sample comes from another part of the country where the soils are undoubtedly highly fertile.

The conclusions which can be drawn from these analyses are that there is a marked shortage of boron in some of the plants grown at Kawetire and that zinc may be in short supply while all other

TABLE 2

Further analyses * of Mbeya pine leaves with an analysis of flourishing pine leaves from another area for comparison

Sample	Site	N	P	K	Ca	Mg	S	Mn	Fe	Cu	B	Zn
5	Kawetire (showing dieback)	1.00	**.05**	.32	.38	.12	.34	237	413	n.d	**18**	n.d
6	Kawetire (unhealthy)	1.48	.10	.38	.40	.11	.33	226	206	n.d	**8**	n.d
7	Mbeya Nursery (healthy)	1.79	.16	1.07	.57	.25	.26	227	310	n.d	25	n.d
8	Moshi (flourishing)	1.95	.13	.38	.41	.11	.26	281	225	n.d	53	n.d

* The first six columns – N to S in Tables I and 2 are as %, the remainder as parts per million total content of element in leaf dry matter; nutrient levels which are suspect have been printed in heavy type; n.d = not determined.

essential plant nutrients (except P in No. 5) are probably adequate. Healthy dicotyledenous plants usually contain at least 20 ppm B in their leaves, and published data on some 50 spp. average about 50 ppm. None of the Mbeya-Kawetire samples are at all well supplied with boron by Moshi or general plant standards.

FIELD EXPERIMENTS

On the basis of the indications given by these leaf analyses, a number of field experiments were carried out a Kawetire.

Experiment 1

This experiment was started in 1955 and consisted of 5 adjacent rows of 20, four-year-old trees; row treatments being NPK, NP, NK, PK and Nil at doses of 1 ounce per tree of sulphate of ammonia, single supperphosphate, and muriate of potash. B-plus-Zn spray was applied to half of each row, the spray being 1% zinc sulphate, 0.25% borax and 0.5% lime with sticker and spreader. Two sprayings were given – one in September and one in November.

By May 1956 dieback in the sprayed portion of the experiment was being overcome and healthy leading shoots had been thrown up while in the unsprayed portion only two trees had recovered. Further observations in July 1956 confirmed the improvement in the sprayed portion and none of the sprayed trees had any

signs of dieback. By September 1957, the untreated trees outside the experiment had overcome the dificiency and were growing satisfactorily. The B-plus-Zn spraying in the experiment had eliminated the set-back to growth which would otherwise have occurred.

Experiment 2

In this experiment comparable unthrifty two-year-old trees of two species – *Pinus patula* and *P. radiata* – were selected and divided into 4 groups, on a scattered layout, for control (water), B, Zn, B-plus-Zn spraying. Each treatment was applied to 15 trees of each species. Spraying was done three times at 3-monthly intervals starting in February 1958. The boron sprays gave a significant improvement in height increment between March and October 1958. (Table 3).

TABLE 3

Height increment in inches caused by Fertilizer treatment		
Treatment	*P. patula*	*P. radiata*
Control	4.8	8.4
Boron	7.1	14.0
Zinc	5.9	6.7
Boron plus zinc	6.3	12.0
Significant difference	2.3	3.8

The number of trees showing dieback in this experiment in January 1959 is shown in Table 4.

TABLE 4

Number of trees showing dieback as influenced by fertilizer treatment		
Treatment	*P. patula*	*P. radiata*
Control	6 trees	4 trees
Boron	1 tree	1 tree
Zinc	6 trees	7 trees
Boron plus zinc	2 trees	0 trees

Combining both species the incidence of dieback becomes: – Without boron – 23 out of 60 trees, and with boron – 4 out of 60 trees. This difference is highly significant and confirms that the dieback is due to boron deficiency.

Experiment 3

This experiment was planned to test soil application of boron and zinc because of the impracticability of using sprays on a large scale in the afforestation areas.

The layout was a randomised block of 5 treatments × 6 replications, each replication being a row of 10 one-year-old plants. Treatments were control, B, Zn, B plus Zn, and a trace element frit. Boron and zinc doses were 1/10 ounce each per plant of borax and zinc sulphate respectively and the frit was applied at $\frac{1}{4}$ ounce per plant. The experiment was laid down in March 1958. Mean heights in inches in November 1959 were as follows: –

Heights in inches as influenced by fertilizer treatments		
Treatment	*P. patula*	*P. radiata*
Control	43.31	66.65
Boron	62.05	78.40
Zinc	49.71	64.36
Boron plus zinc	64.10	76.52
Frit	48.75	62.69

The growth responses to treatments containing boron are significant in both species. No dieback was noticed in this experiment which indicates that shortage of boron retards growth before deficiency symptoms appear. The frit used in this experiment was a commercial product containing a range of trace elements including B. Its failure to give responses is possibly due to the low solubility of the boron it contains.

This experiment concluded the diagnostic stage of the investigation and some 600 acres of pine plantings are now being treated with borax at the rate of 15 pounds per acre as a soil application.

It is clear that boron shortage is rather widespread in the Southern Highlands Province of Tanganyika; deficiencies having also been detected in wattle [1], coffee [2], maize [3] and pyrethrum [3].

SUMMARY AND CONCLUSIONS

Leaf analyses have indicated that the dieback investigated was due to boron deficiency and this has been proved by field experiments. Incidence of boron deficiency is tentatively related to the weathered condition of the soils on which it occurs and a climatic regime which may be alternately

too wet and too dry for optimum availability of soil boron. The best means of correcting the deficiency appears to be the application of a small amount of borax – 15 pounds per acre – to the soil towards the end of the rainy season.

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